STUDIES OF CHEMICAL AND BIOLOGICAL PROCESS EFFECTS OF INDUSTRIAL WASTES RELEASED INTO THE GULF OF MEXICO BY OCEAN DUMPING -- DRAFT FINAL REPORT

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Report for NOAA on Ocean Dumping in the Gulf of Mexico

Chemical Characterization

by

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Introduction

The goal of this project was to identify and quantify selected chemicals, trace metals and organic molecules, in Material to be Dumped (MTBD). The MTBD was provided by NOAA and included substances which were suspected of being deleterious to marine life. In addition studies of the $^{13}C/^{12}C$ ratio, $\delta^{13}C$, of the total organic matter of the samples were done in order to evaluate the potential of $\delta^{13}C$ as a tracer for Dumped Material as it mixes with the dissolved and particulate organic matter of the ocean.

The experimental plan had been to isolate the toxic fraction of MTBD in cooperation with the biologists, finally identifying the exact molecule(s) that were toxic. This proved to be beyond the scope of the research project because of the complexity of the MTBD. The results obtained point to the general problem of regulating ocean dumping, that is that the chemical composition of the MTBD is highly complex and highly variable with source - further the toxicity of MTBD for open ocean organisms is itself highly complex and variable.

The chemical studies were divided into three tasks:

A. $\delta^{13}C$ of particulate and dissolved organic matter in MTBD.

B. Isolation and identification of selected organic compounds in MTBD. This study includes GC and GC/MS studies of the lipid soluble fraction of the MTBD.

C. Concentration of selected trace metals in the MTBD.

The Materials to be Dumped (MTBD) were:

Shell biosludge # 1, received February 23, 1977
Shell biosludge # 2, received June 21, 1977
Shell biosludge # 3, received November 9, 1977

A. $\delta^{13}C$ of Material to be Dumped (MTBD)

The $\delta^{13}C$ value of organic material has proven to be a useful tracer in pollution studies (Calder and Parker, 1968), food chain studies (Parker et al., in press) and in geochemistry (Fry, et al., 1977). It seemed worthwhile to investigate the potential of using $\delta^{13}C$ as a tracer for the mixing of dumped material with the dissolved (DOC) and particulate (POC) organic matter of the sea. The $\delta^{13}C$ value of DOC and POC are close to -20 on the PDB scale. If dumped organic matter is significantly different (i.e. several units) then mixing may be traced.

The $\delta^{13}C$ results, measured and literature, are summarized in the following data:

	$\delta^{13}C$*	Typical Conc. in the Gulf.
Shell sludge, particulate (SS-POC)	-24.9	‡
Puerto Rico effluent, dissolved (PR-DOC)	-25.5	#
Sediment carbon (SOC)	-19.	1%
Dissolved carbon (DOC)	-20.2	1 ppm
Particulate carbon (POC)	-20.4	0.5 ppm

* Units are per mil vs. PDB carbonate std.

‡ EC is 16% organic carbon

Puerto Rico effluent was not a part of the project, but was run to test the concept of a tracer. The P-DOC level is 14,600 ppm.

The interesting point in this data is the sharp resolution of $\delta^{13}C$ of the SS-POC and PR-DOC from the natural carbon reservoirs of SOC, DOC and POC. Since the error in these $\delta^{13}C$ values are $\pm$ 0.3 the 4 to 5 per mil resolution may be used to carryout model mixing calculations. Calder and Parker (1968) showed that the ratio of carbon derived from pollutant to natural carbon is a function of the $\delta^{13}C$ values of the three reservoirs:

$$\frac{C_p}{C_n} = \frac{\delta_n - \delta_n}{\delta_m - \delta_p} \qquad (1)$$

where: C_p = moles carbon derived from pollutant

C_n = moles natural carbon

δ_n = $\delta^{13}C$ of natural carbon

δ_p = $\delta^{13}C$ of pollutant carbon

δ_m = $\delta^{13}C$ of any mixture of natural and pollutant carbon,

note that the ratio C_p/C_n has no units.

In the case of mixing Shell sludge (SS-POC) with natural POC equation (1) becomes:

$$\frac{C_{SS - POC}}{C_{nat. - POC}} = \frac{\delta_n - \delta_m}{\delta_m - \delta_s} \qquad (2)$$

where δ_s = $\delta^{13}C$ of SS-POC and the others remain as in (1).
It can be seen that a value of $C_{SS}/C_{nat.}$ = 1 (i.e. a 50-50 mixing of pollutant and natural carbon) will result if only 3 mg of Shell sludge is added to a litre of sea water. The value of δm would be -22.8 which could be resolved from -20 since the error is 0.3. Equation (2) is very sensitive to $C_{nat.}$, the concentration of natural

POC; if it is less than 0.5 ppm as it probably would be in open water then the tracer works even better.

The $\delta^{13}C$ value of the pollutant DOC of the Puerto Rico effluent is very favorable to a tracer study. It is estimated that the tracer method would detect the addition of 1 ml of the effluent to 1 litre of seawater. Further the subsequent mixing and, biodegradation (if any) and bio-uptake of the effluent could be detected by the $\delta^{13}C$ method.

Our conclusion is that $\delta^{13}C$ offers good prospects as a tracer for particulate and dissolved dumped organic matter in the natural POC and DOC reservoirs. It does not appear useful for sediment because the natural sediment reservoir is too large and too distant for necessary conditions.

B. Qualitative Analysis of Organic Components of "Biosludge" Materials

Analytical procedures used in these studies are qualitative and no attempt has been made to quantify the methods. This is primarily because of the complex nature of the materials and the variability between samples. Two samples have been processed: 1) "Sample II"; and 2) "Sample III (9 Nov '77)".

The extraction and analysis procedure for both samples was identical and is represented schematically in Fig. 1. Three fractions were prepared from each sample by a three-step process of extraction, concentration and fractionation. Each fraction was characterized through application of combined gas chromatography-mass spectrometry-computer data reduction.

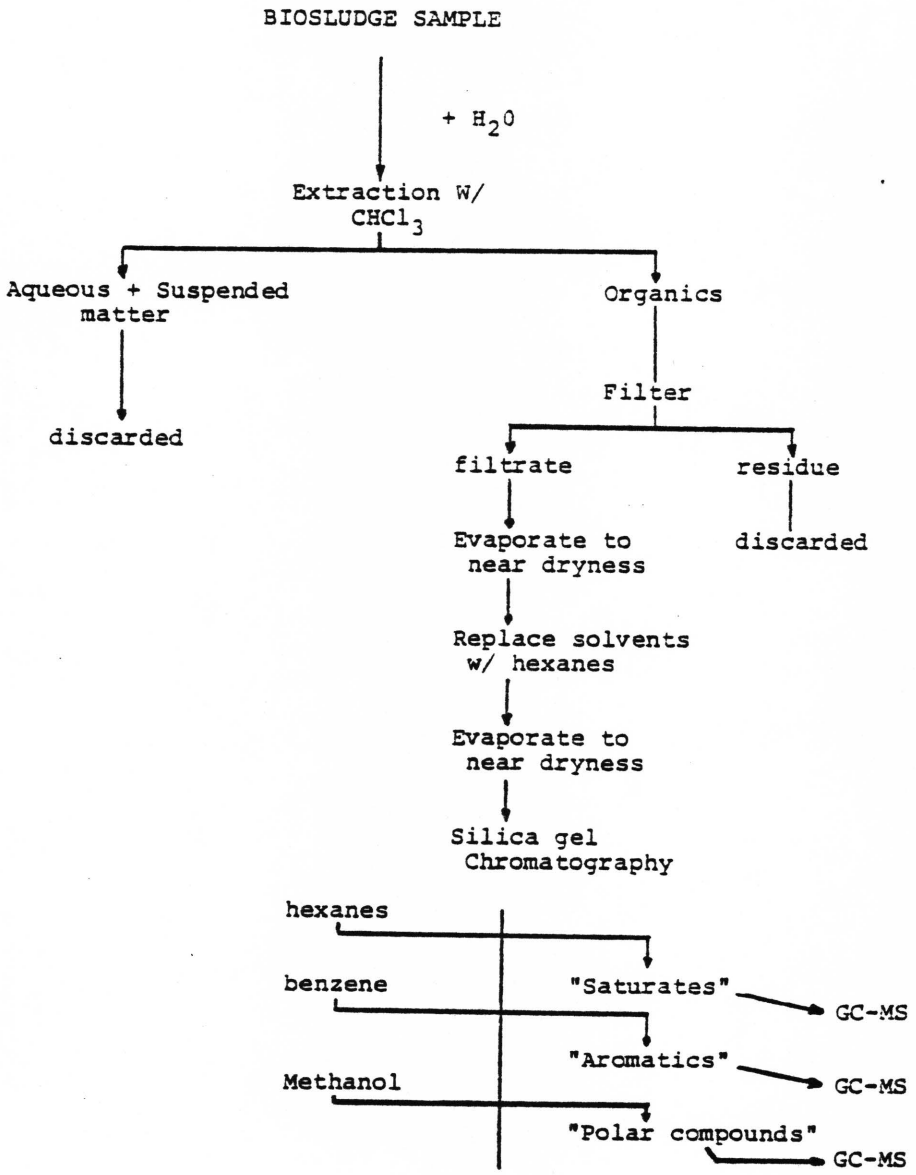

Figure 1. Analytical separation scheme for "Biosludge" samples.

A sample of "biosludge", typically 20 ml, was diluted with distilled-deionized water of equal volume to make it more manageable in a separating funnel. The diluted sample was repeatedly extracted with pure, freshly-distilled chloroform and the aqueous layer with suspended material was discarded. The organic layer was filtered to remove residues and the chloroform solvent was evaporated to near-dryness. The sample extract was taken up in n-heptane and the evaporation procedure repeated. In this manner, the solvent was replaced with heptane. Care was taken to retain volatile compounds by never allowing the sample to evaporate to dryness.

The final extract was diluted to 1 to 2 ml with heptane and was added to the top of a silica gel chromatographic column 20 cm long by 1 cm diameter. Three separate fractions were eluted from the column by successive additions of hexane, benzene, and methanol. The hexane eluates are primarily weakly polar organic compounds such as saturated and some unsaturated hydrocarbons. This fraction is commonly termed the "saturates" fraction. Components eluted with benzene are more polar in nature and include aromatic unsaturated, and multiple ring hydrocarbons and some halogenated and heteroorganic compounds. This is the "aromatic" fraction. The methanol-eluted fraction contains highly polar compounds such as heterocyclic organics, alcohols, some complex halogenated compounds, etc.

The three fractions were characterized using a combined gas chromatograph-mass spectrometer-computer data system. The instrument used in this study is a DuPont Model 21-491 GC-MS with

a Model 21-094B data system. The chromatographic column was 1/8 inch diameter, 6 foot long stainless steel packed with 5% FFAP stationary phase on Gas Chrom-Q 80 - 100 mesh. The column was temperature programmed from 70°C to 260°C at 6°C per minute using helium as a carrier gas. The mass spectrometer jet-separator and ion source were operated at 200°C, ionization potential was 70 ev, and ion acceleration potential was approximately 1300 volts. The magnetic sector was continuously scanned at 4 "decades per second" from mass 517 down to mass 41. Mass spectral data were continuously acquired and stored on magnetic disc for each scan. Data reduction is accomplished through computer software furnished by DuPont as modified in our laboratory.

Sample II (June 21, 1977)

Hexane, benzene and methanol eluates from silica gel column chromatography were concentrated to equal volumes and submitted to gas chromatographic analysis. Figure 2 illustrates the results of analyses of equal injection volumes of the three fractions. The largest portion of extractable organics was present in the methanol eluate, or polar fraction, Figure 2C.

Saturate fraction (hexane eluate)

The largest component of the saturate fraction was elementary sulfur which precipitated during concentration. No mass spectral data was obtained for this fraction.

Aromatic fraction (benzene eluate)

The reconstructed gas chromatogram (total ion chromatogram) of the aromatic fraction is given in Figure 3. Mass spectra for

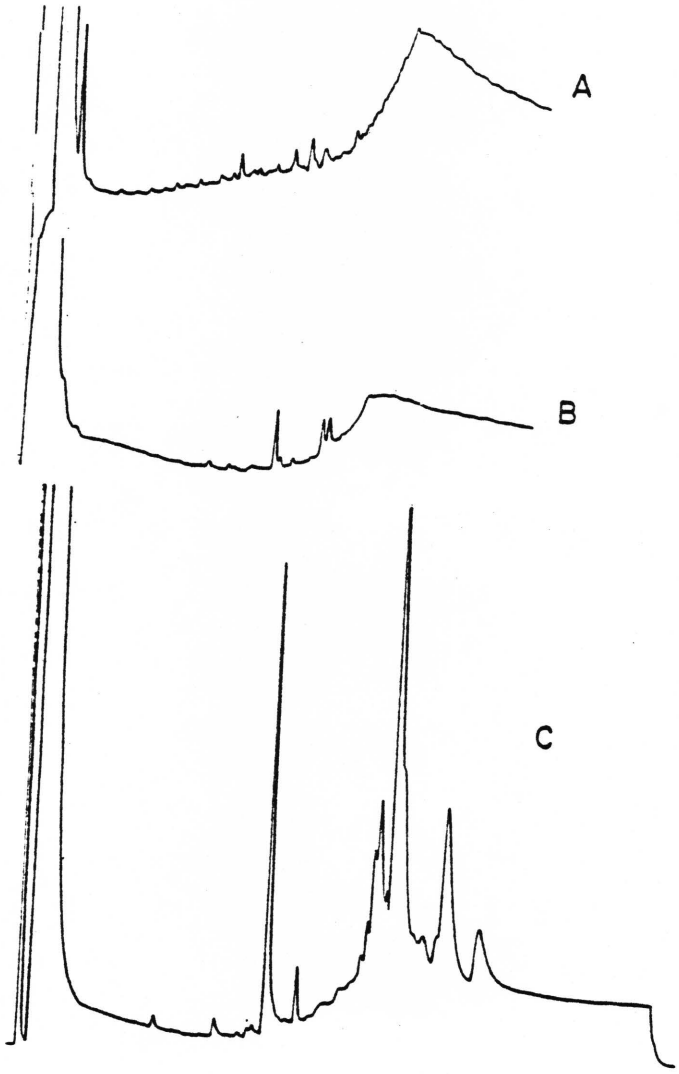

Figure 2. Gas chromatograms of saturate (A), aromatic (B) and polar compounds (C) fractions of Sample II.

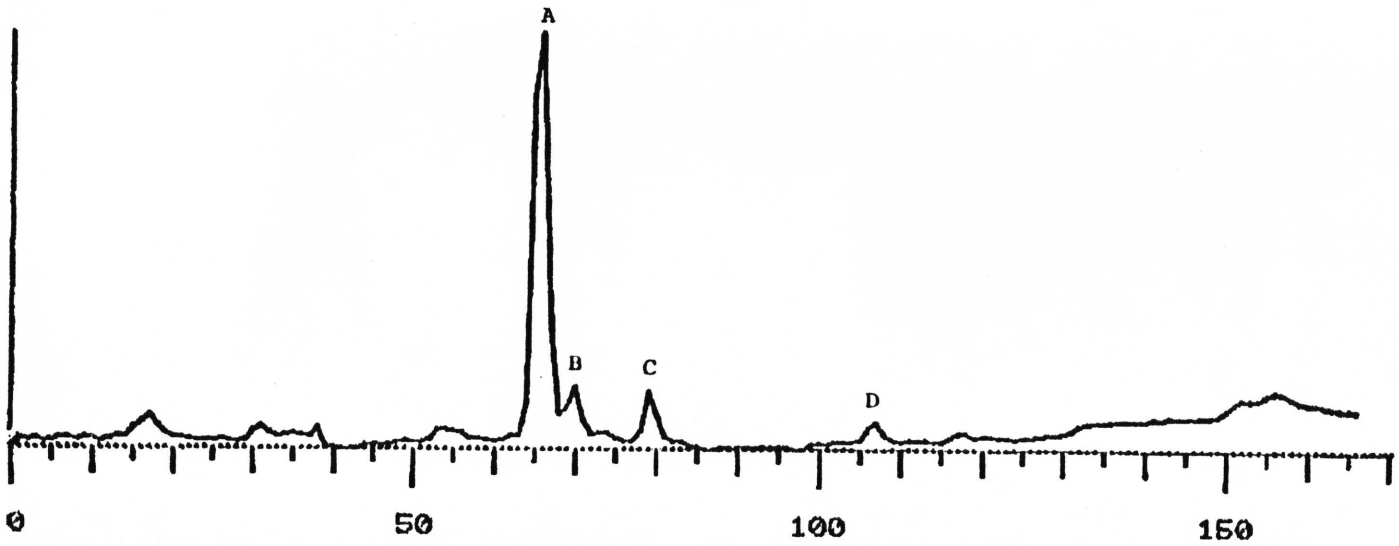

Figure 3. Reconstructed total ion gas chromatogram of the saturate fraction, Sample II.

peaks "A-D" of Figure 3 are given in Figures 4 and 5 respectively. Mass spectral data suggests that the first three major components, "A-C", are chlorinated compounds which contain nitrogen. The fourth compound, "D", has been tentatively identified as a methyl-indole probably primarily 3-methyl-indole, skatole. Skatole is a well known product of the metabolism of nitrogen compounds by bacteria.

The presence of trace amounts of aromatic hydrocarbons in this fraction were indicated by mass chromatograms. Naphthalenes, phenanthrene and/or anthracene and alkyl derivatives of these compounds were detected by mass chromatograms such as those given in Figures 6 and 7.

Polar compound fraction (methanol eluate)

The reconstructed gas chromatogram of the polar compound fraction is given in Figure 8. Mass spectra from the major components labeled "E-L" are given in Figures 9-12. The largest component of the fraction, "E", has been tentatively identified as a cresol (methyl phenol). The gas chromatographic retention time of the cresol is identical to that of authentic meta and para cresol which coelute on this column. The ortho isomer was not present.

Components "F" and "G" have mass spectra which suggest they are halogenated. Component F has an apparent molecular weight of 217 which would indicate by its odd mass that the compound probably contains an odd number of nitrogen atoms.

Components "H", "I", "J" and "L" are tentatively identified as free fatty acids with molecular weights of 228, 242, and 256

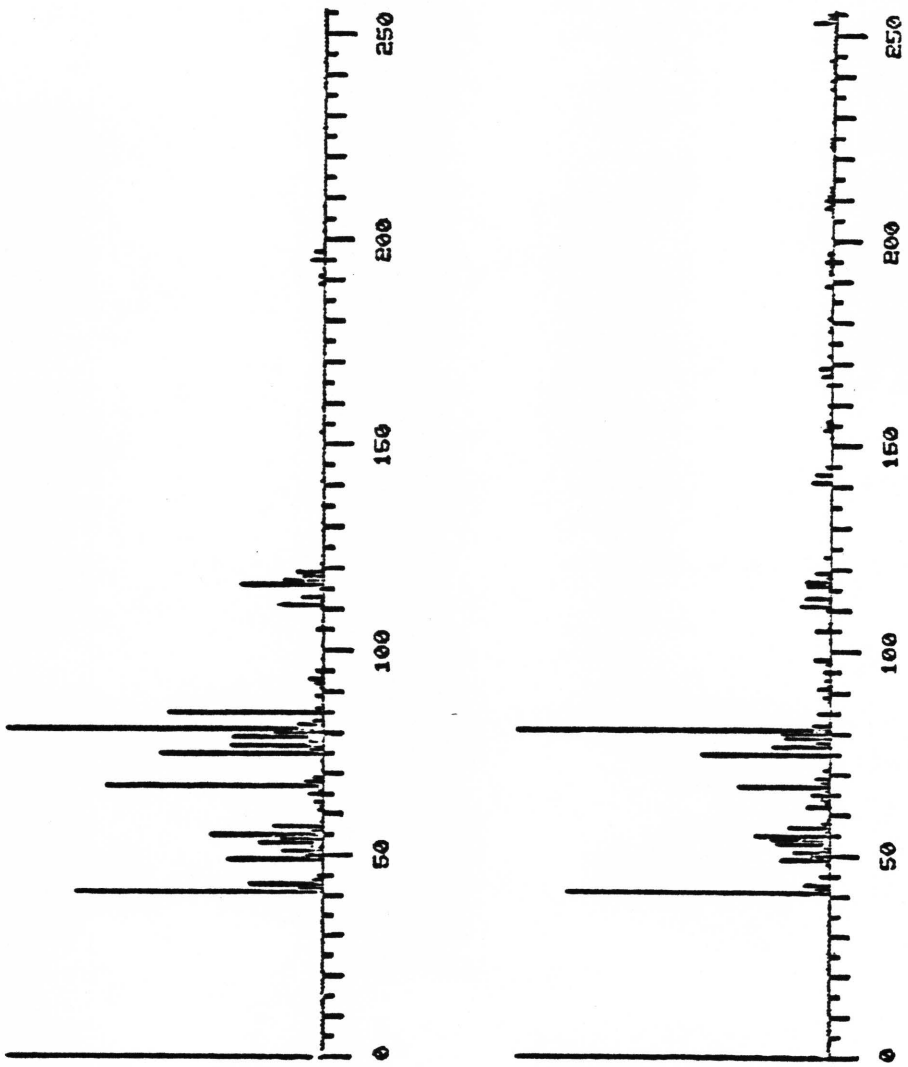

Figure 4. Mass spectrum of component "A" above, "B" below.

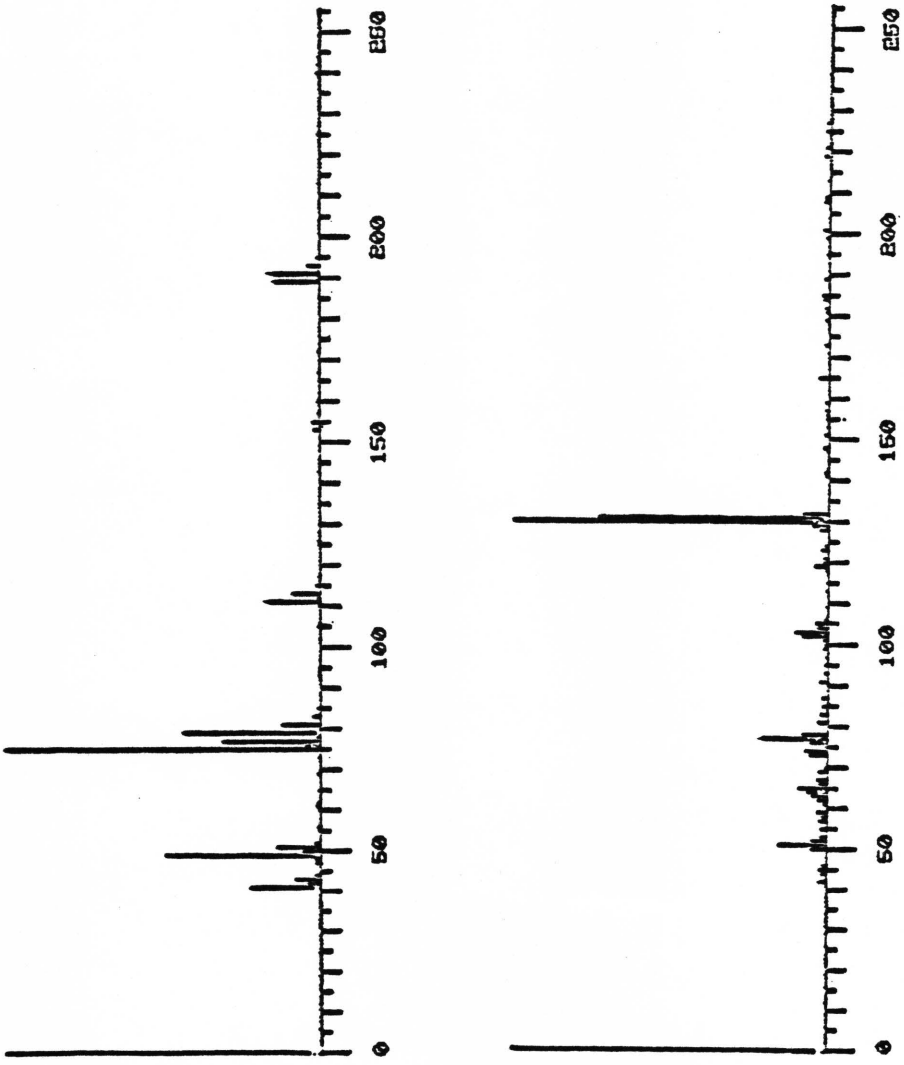

Figure 5. Mass spectrum of component "C" above; "D", methylindole, below

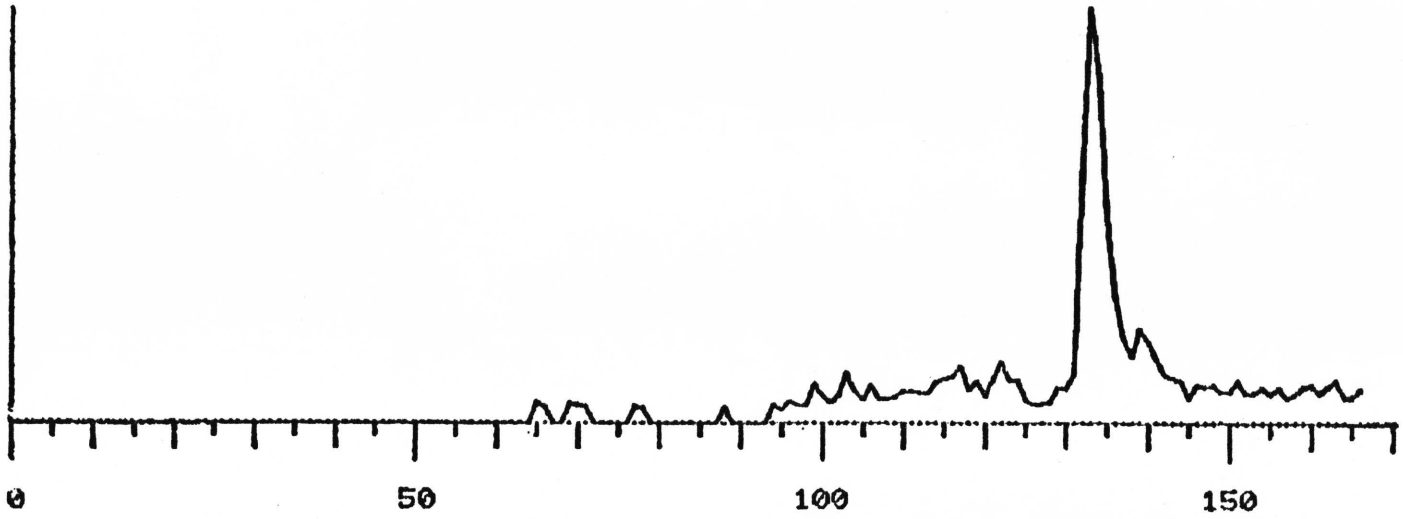

Figure 6. Reconstructed mass chromatogram of benzene eluate at mass 178, phenanthrene.

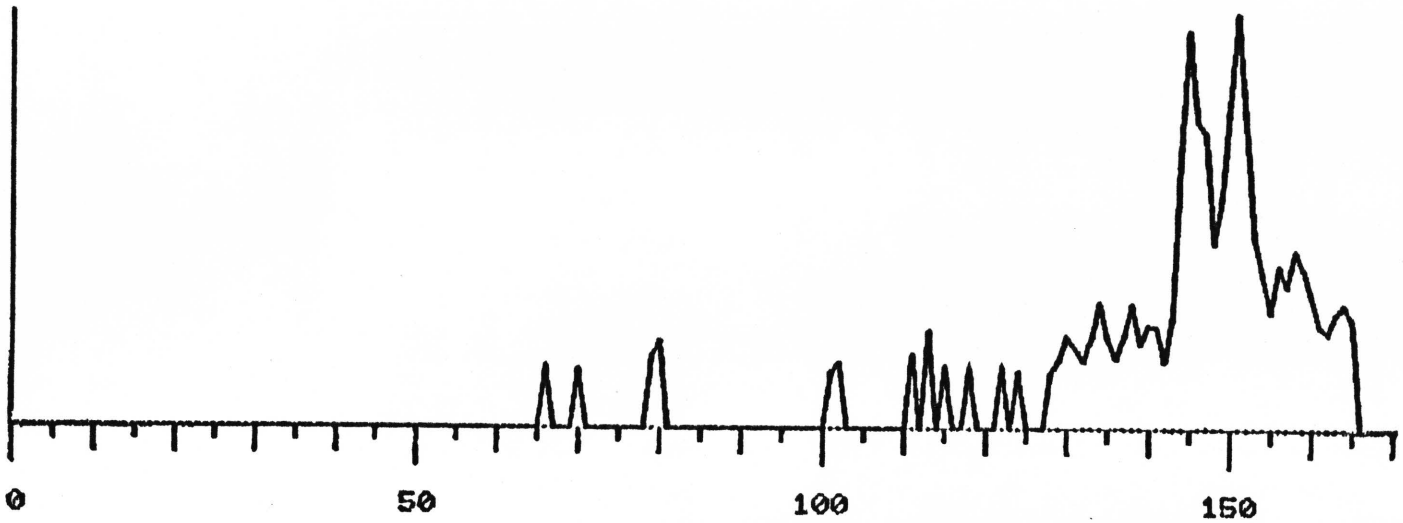

Figure 7. Reconstructed mass chromatogram of benzene eluate at mass 192, methylphenanthrene.

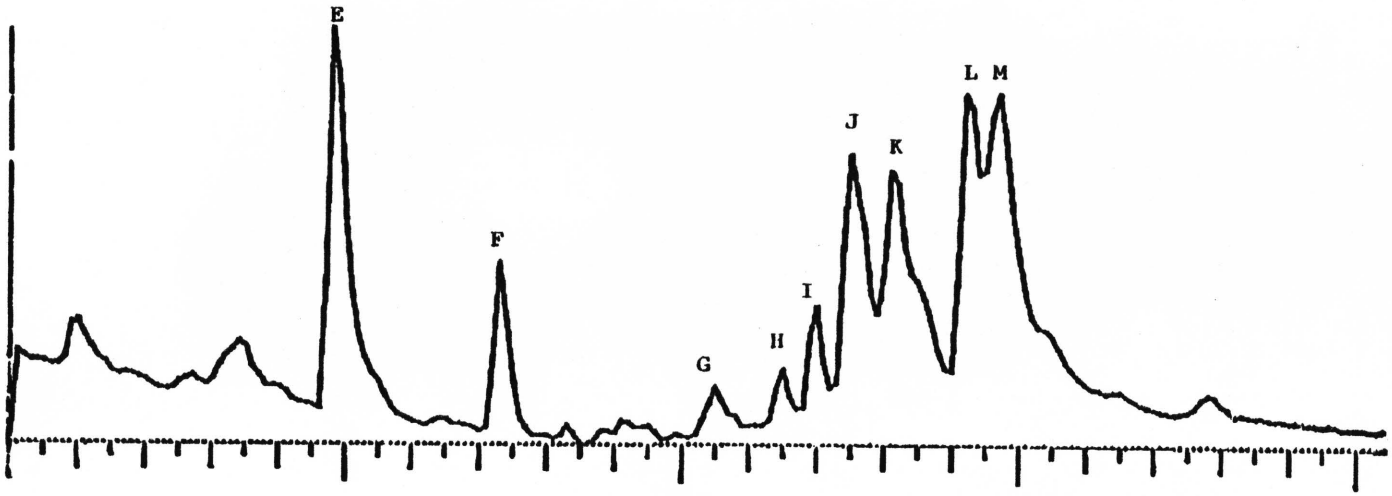

Figure 8. Reconstructed total ion gas chromatogram of the polar compound fraction, Sample II.

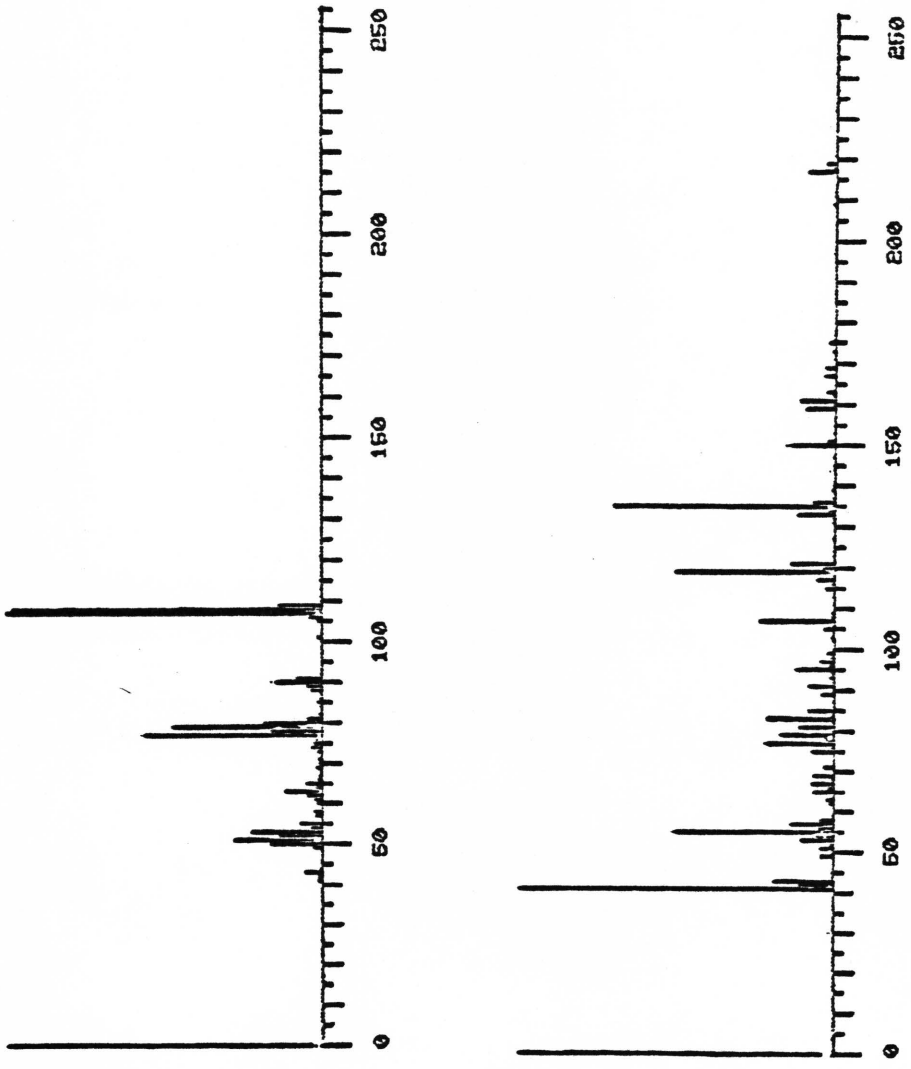

Figure 9. Mass spectrum of component "E", cresol, above; "F" below.

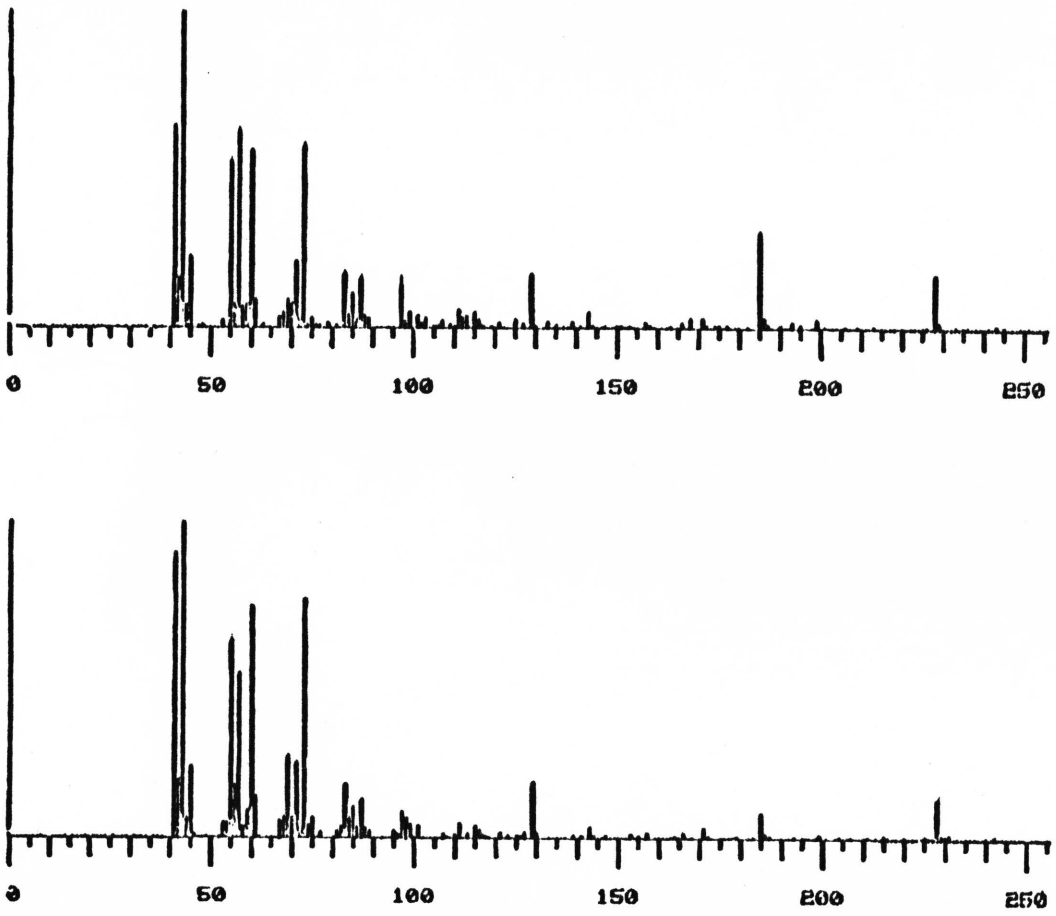

Figure 10. Mass spectrum of component "H", a C_{14} fatty acid, above; "I" also a C_{14} fatty acid, below.

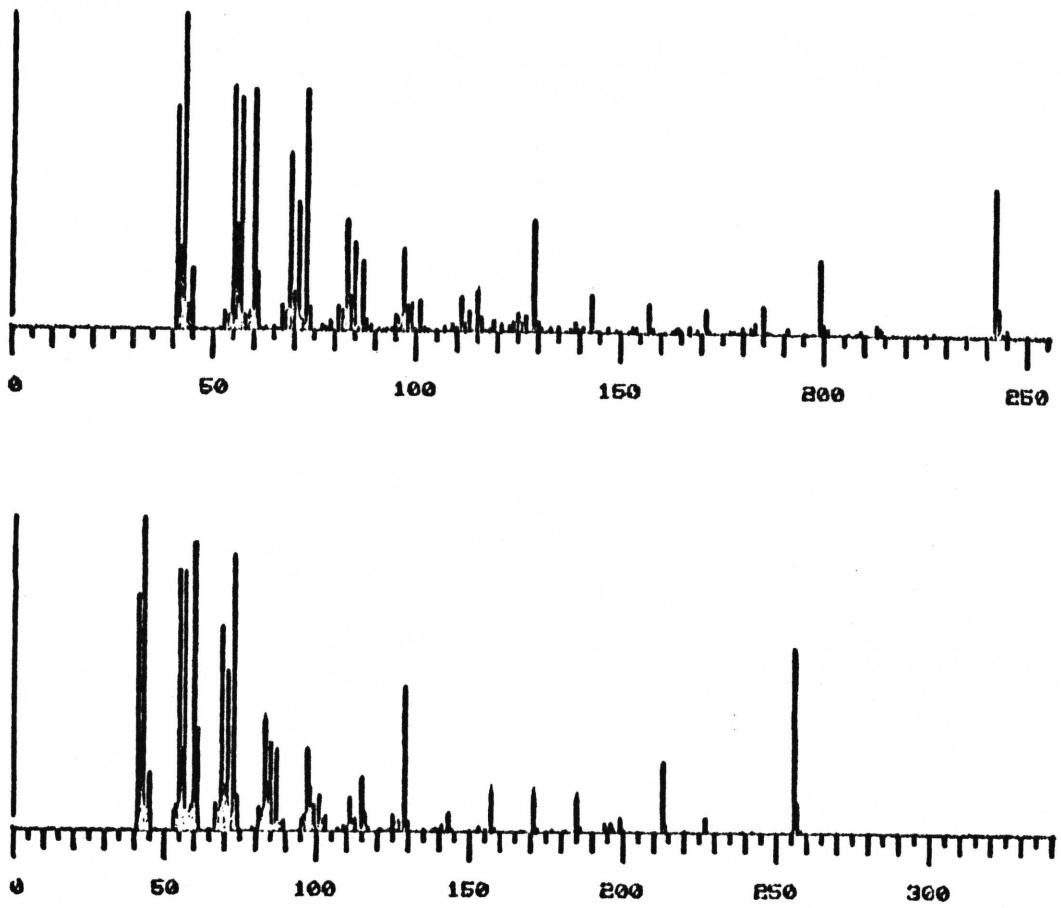

Figure 11. Mass spectrum of component "J", a C_{15} fatty acid, above; "L" a C_{16} fatty acid below.

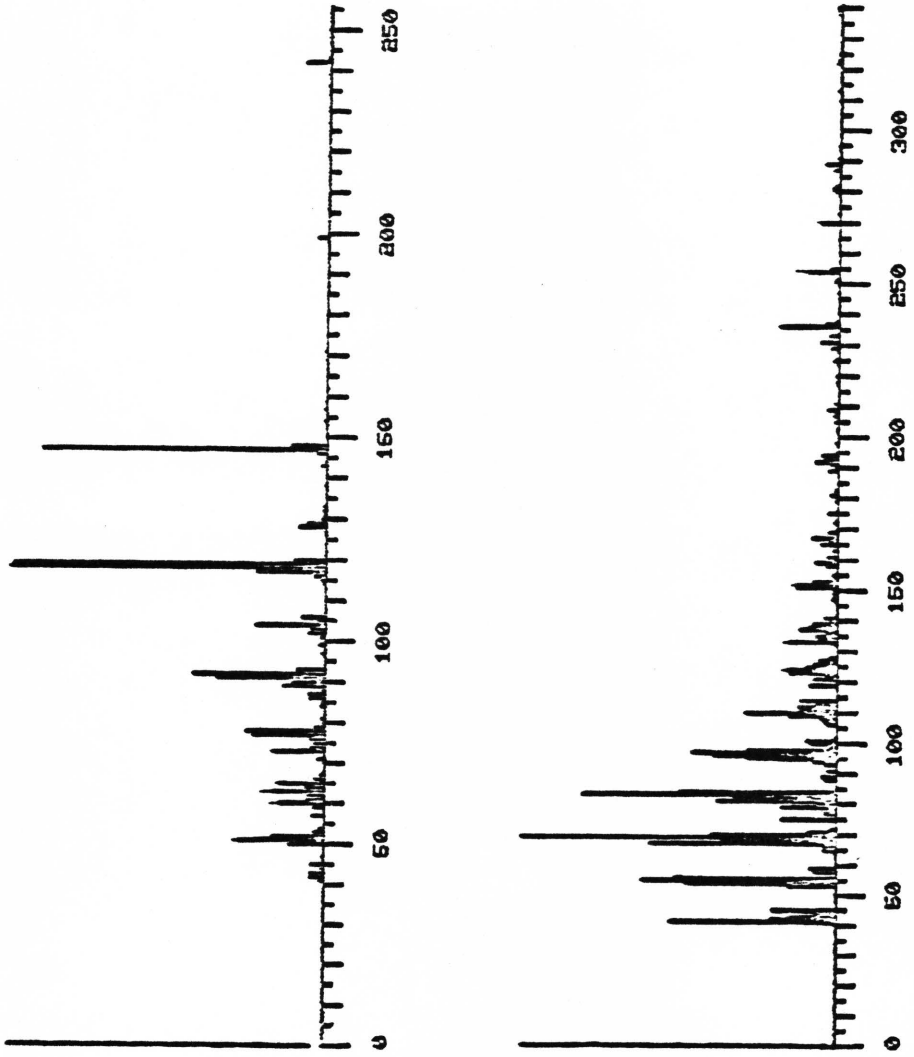

Figure 12. Mass spectrum of component "K" above, "M" below.

which correspond to C_{14}, C_{15} and C_{16} acids.

Component "M" has an apparent molecular weight of 254, contains no halogens, and probably is an alcohol (strong M^+-18 peak at 236).

Component "K" with an apparent molecular weight of 147 probably contains one nitrogen atom and a phenolic hydroxy group.

Sample III (November 9, 1977)

Saturate fraction

The total ion chromatogram for the saturate fraction is given in Figure 13. The chromatogram is characterized by an unresolved mixture of compounds in the range of scans 150 to about 300. A few resolved compounds are observed, the most prominent of which is labeled "A" in Figure 13. The mass spectrum of this component, given in Figure 14, is typical of long chain n-alkanes and in this case the molecular ion peak at M/e = 240 indicates the component is n-heptadecane (n - $C_{17}H_{36}$). A whole series of n-alkanes becomes evident in the mass chromatogram reconstructed from M/e = 85 ions as shown in Figure 15. Most of the peaks in this figure are apparent but less evident in the total ion chromatogram of Figure 13. Peak "A" of Figure 13 corresponds to Peak "A" of Figure 15.

The saturated, branched, isoprenoid alkane, phytane, is identified as component "B" in Figure 15 by the mass spectrum of Figure 16 and its elution position with respect to the normal alkanes. Other isoprenoids are probably present (pristane).

Other homologous series of compounds are also present. An unidentified series having a prominent fragment ion at M/e = 82 is evident in the mass chromatogram shown in Figure 17. The mass spectrum of component "C" is given in Figure 18. The fragmentation pattern resembles compounds containing cyclohexyl groups but the

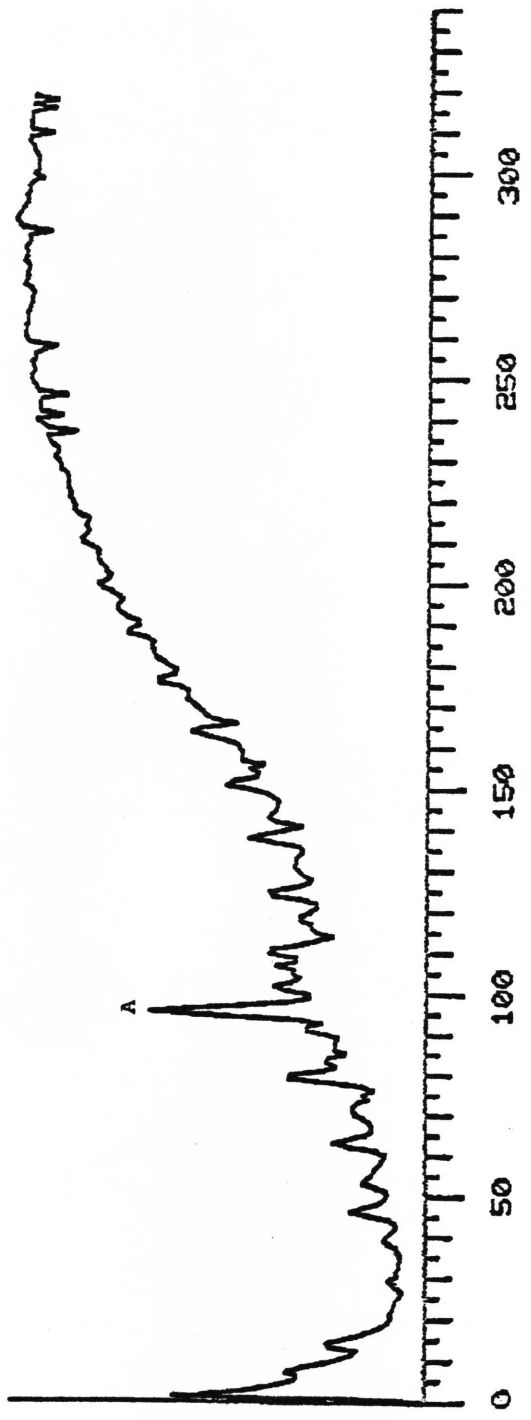

FIGURE 13 RECONSTRUCTED TOTAL ION GAS CHROMATOGRAM OF HEXANE ELUATE FRACTION

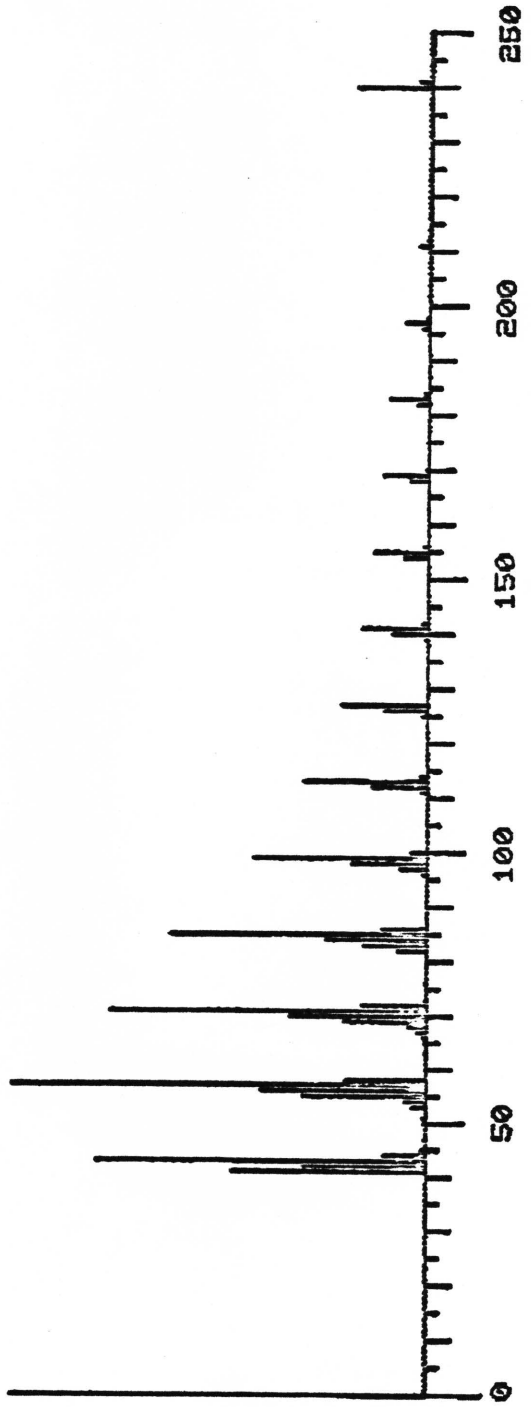

FIGURE 14 MASS SPECTRUM OF COMPONENT "A" OF FIG. 13.

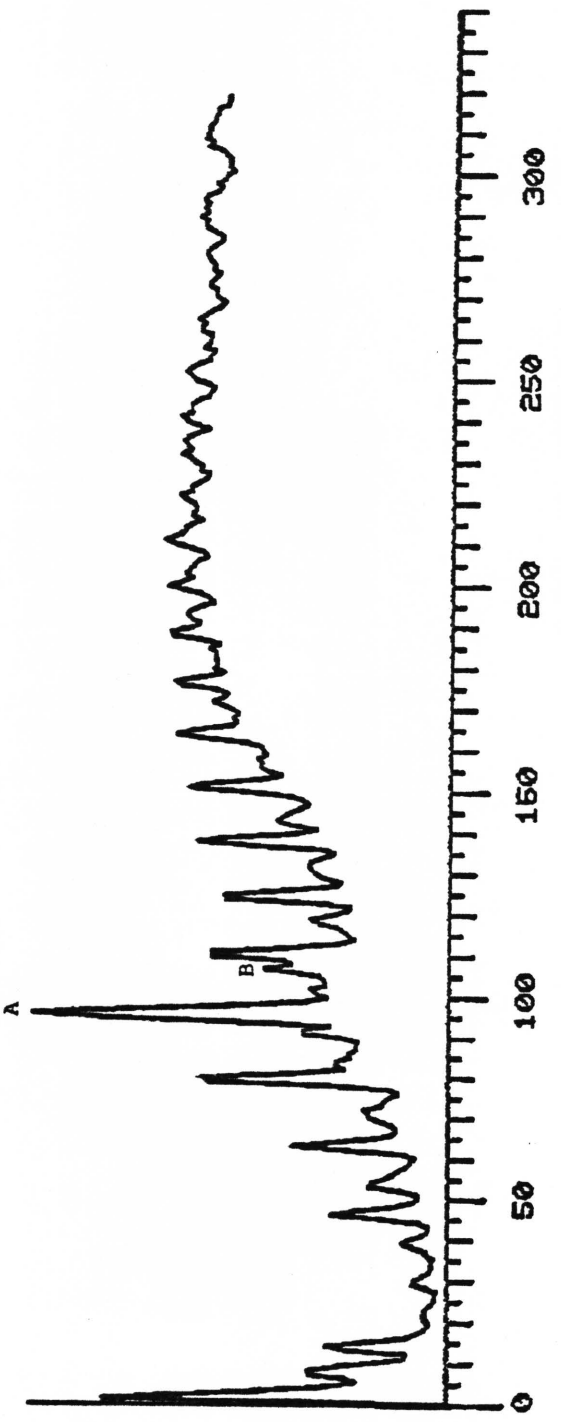

FIGURE 15 RECONSTRUCTED MASS CHROMATOGRAM OF HEXANE ELUATE FRACTION AT MASS 85.

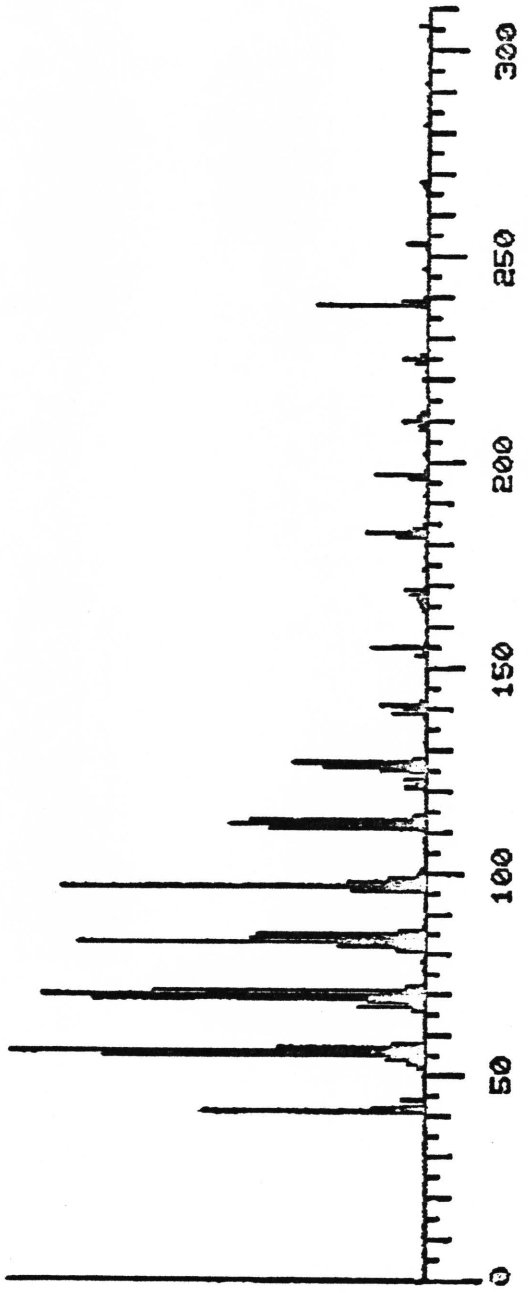

FIGURE 16 MASS SPECTRUM OF COMPONENT "B" OF FIGURE 13.

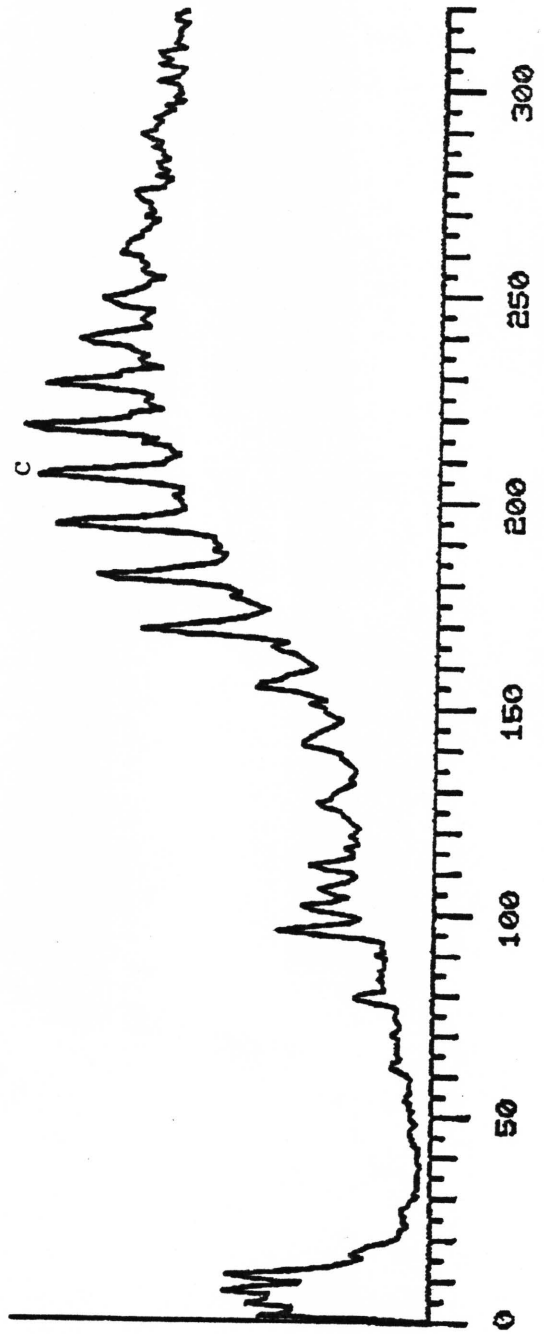

FIGURE 17 RECONSTRUCTED MASS CHROMATOGRAM OF HEXANE ELUATE FRACTION OF MASS 82.

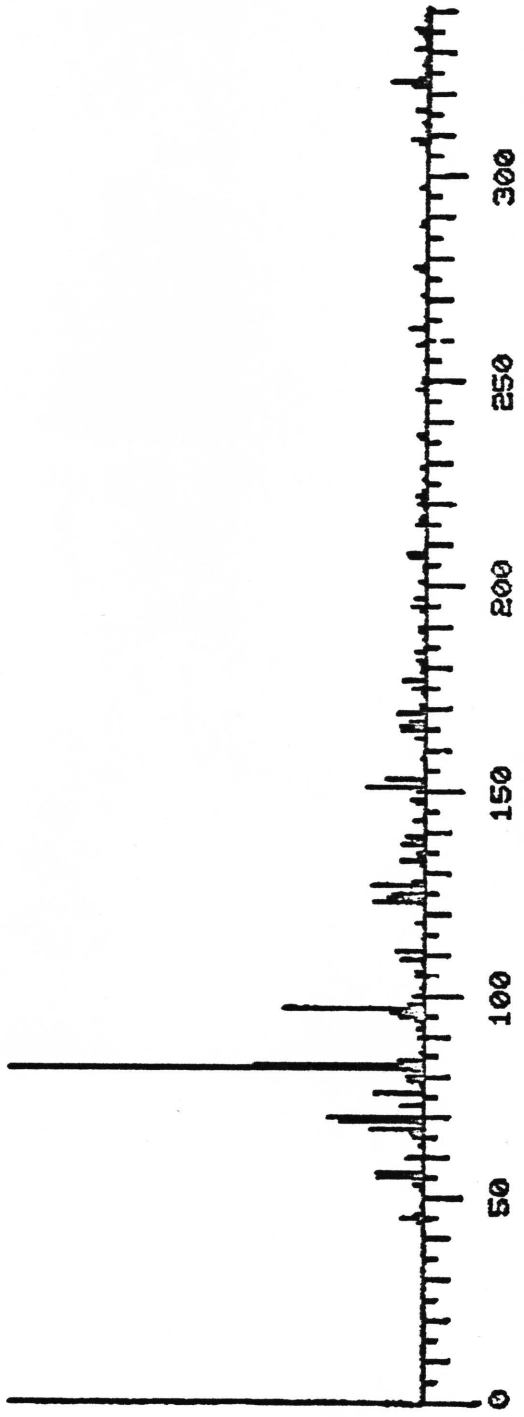

FIGURE 18 MASS SPECTRUM OF COMPONENT "C" OF FIGURE 13.

mass spectra of these relatively minor components are not sufficiently adequate to permit specific identification.

Aromatic fraction

The reconstructed total ion chromatogram for this sample is given in Figure 19 for the "aromatic" fraction. There are three major peaks in this fraction, an incompletely resolved pair at scan 175 and a peak at scan 248. The mass spectra of peaks "D", "E", and "F" of Figure 19 are given in Figures 20, 21 and 22 respectively. These mass spectra are difficult to interpret but all three have characteristics of polyhalogenated compounds. Structure determination on the basis of mass spectrometry alone is probably not possible. Similar mass spectra were not found in a digitized library of 7054 compounds or in a large compendium (Stenhagen et al., 1974). Component "E" appears to be present as a major peak in both Sample II and Sample III.

The "aromatic" fraction also is characterized by a hump of unresolved components. Reconstructed specific ion mass chromatograms are able to resolve some minor components from this hump. Using this technique a component corresponding to naphthalene is resolved in Figure 23.

Peak "H" of Figure 23 has a mass spectrum corresponding to naphthalene and on coinjection into the chromatograph coelutes with naphthalene. Peak "G" also has an identical mass spectrum to that of naphthalene but is not azulene which elutes following naphthalene rather than preceding it. Component "G", which remains unidentified, is also observed in Sample II. Also present in

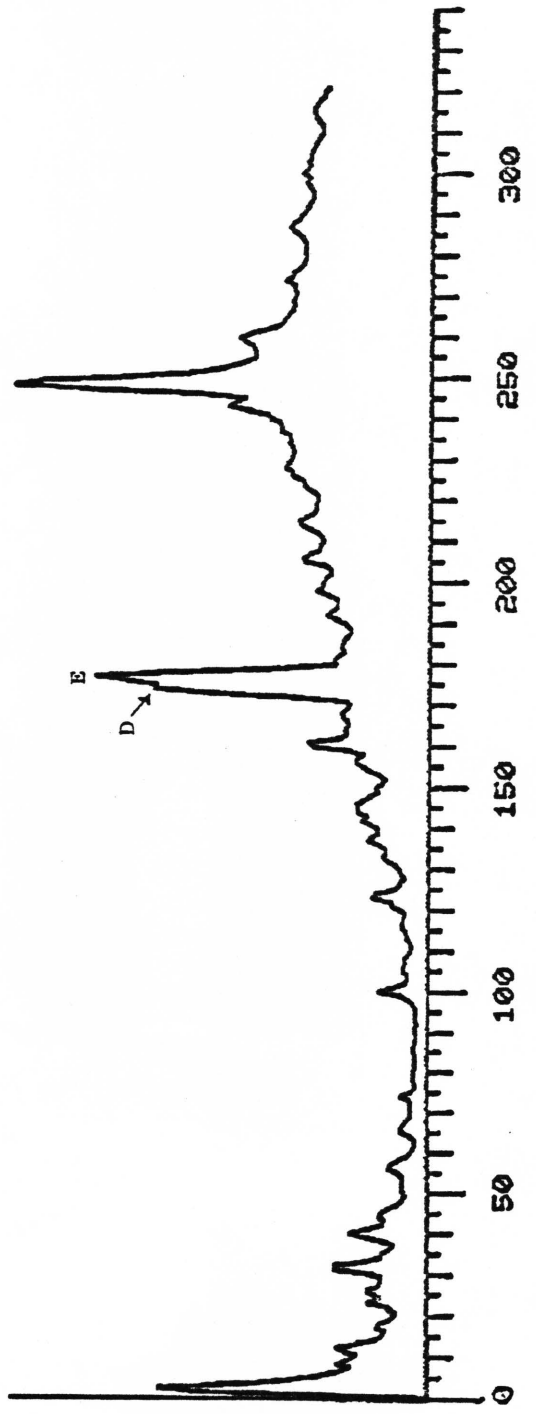

FIGURE 10 RECONSTRUCTED TOTAL ION GAS CHROMATOGRAM OF BENZENE ELUATE FRACTION.

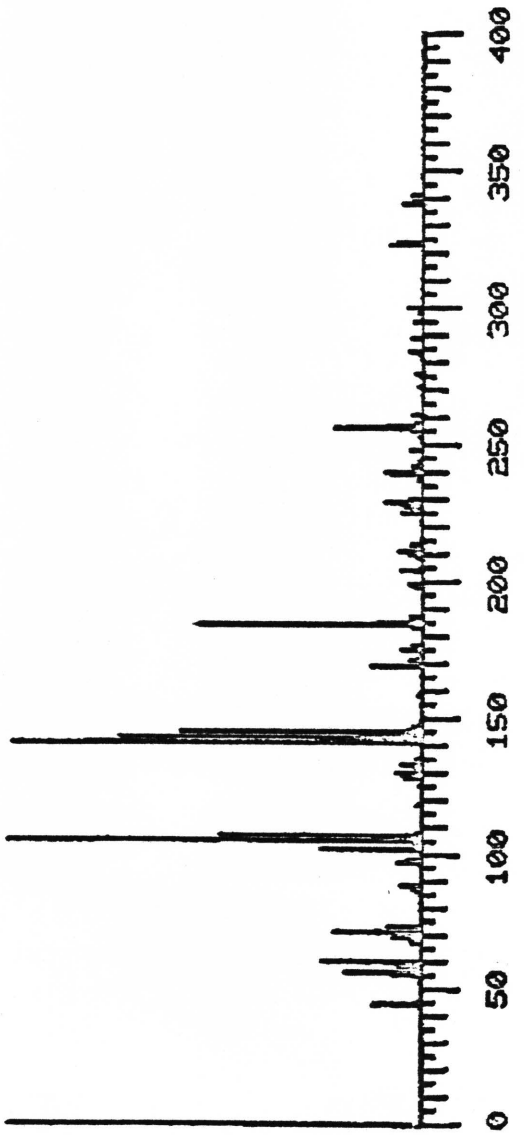

FIGURE 20. MASS SPECTRUM OF COMPONENT "D" OF FIGURE 19.

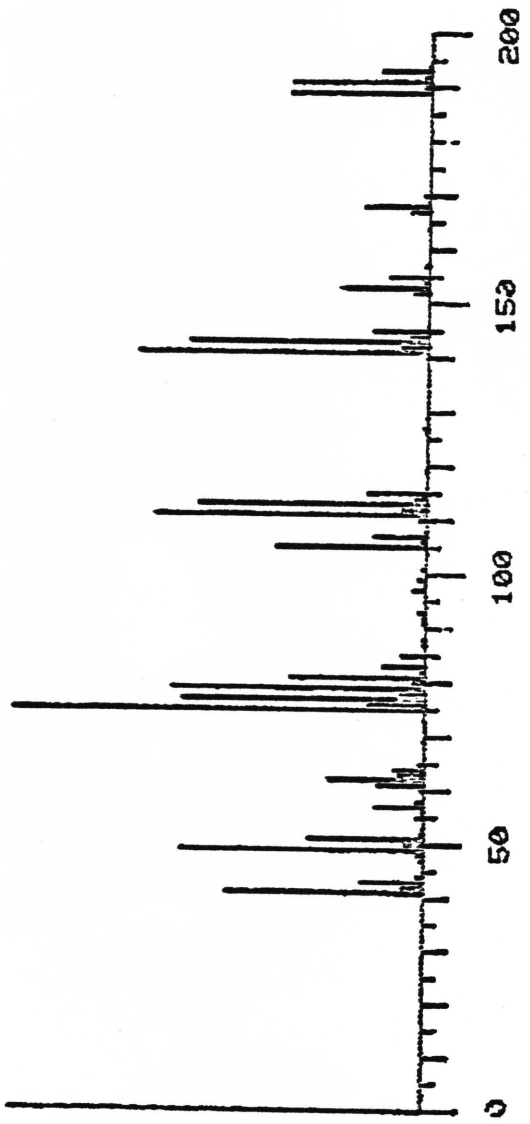

FIGURE 21 MASS SPECTRUM OF COMPONENT "E" OF FIGURE 19.

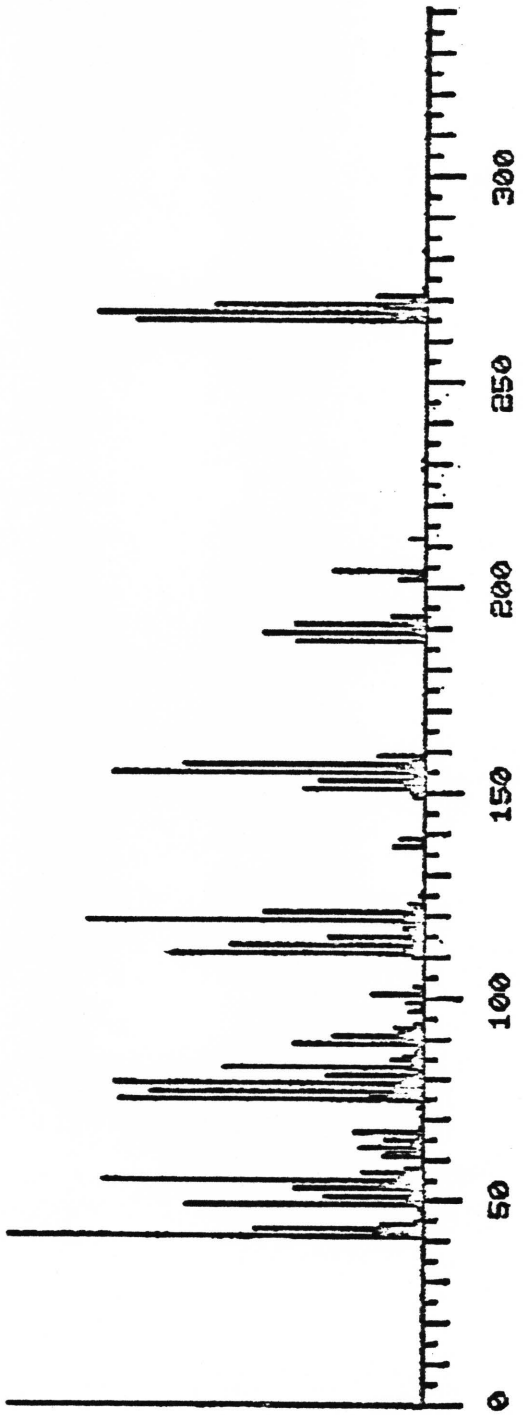

FIGURE 22 MASS SPECTRUM OF COMPONENT "F" OF FIGURE 19.

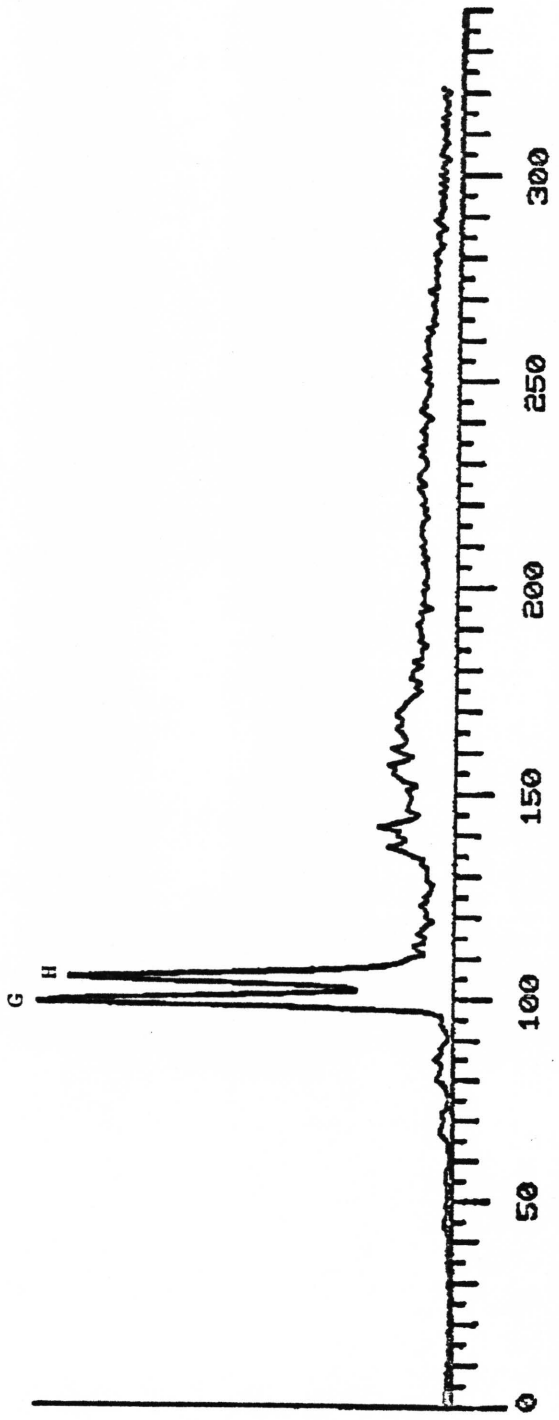

FIGURE 23. RECONSTRUCTED MASS CHROMATOGRAM OF BENZENE ELUATE FRACTION AT MASS 128.

Sample III are the parent and methylated homologs of polynuclear aromatic compounds such as naphthalenes, fluorenes, phenanthrenes (anthracenes), etc. All of these components are present in relatively minor concentrations.

Polar compound fraction

The reconstructed total ion chromatogram for the methanol eluate fraction is given in Figure 24. The major component, peak "J", is apparently an aromatic acid of molecular weight 136. A methyl substituted benzoic acid is likely. The mass spectrum of this peak is given in Figure 25.

Peak "I" has been identified as triethyl phosphate. The mass spectrum is given in Figure 26. Alkyl phosphates are common alkylating agents and fuels and lubricants additives. Peak "K" is unidentified.

Some other minor components can be identified in this fraction. Phenol is readily observed at scan 145 (Figure 27) and a cresol at scan 156 (Figure 28). Neither component is present in sufficient quantity that it is observed in the total ion chromatogram (Figure 24) as a discrete peak.

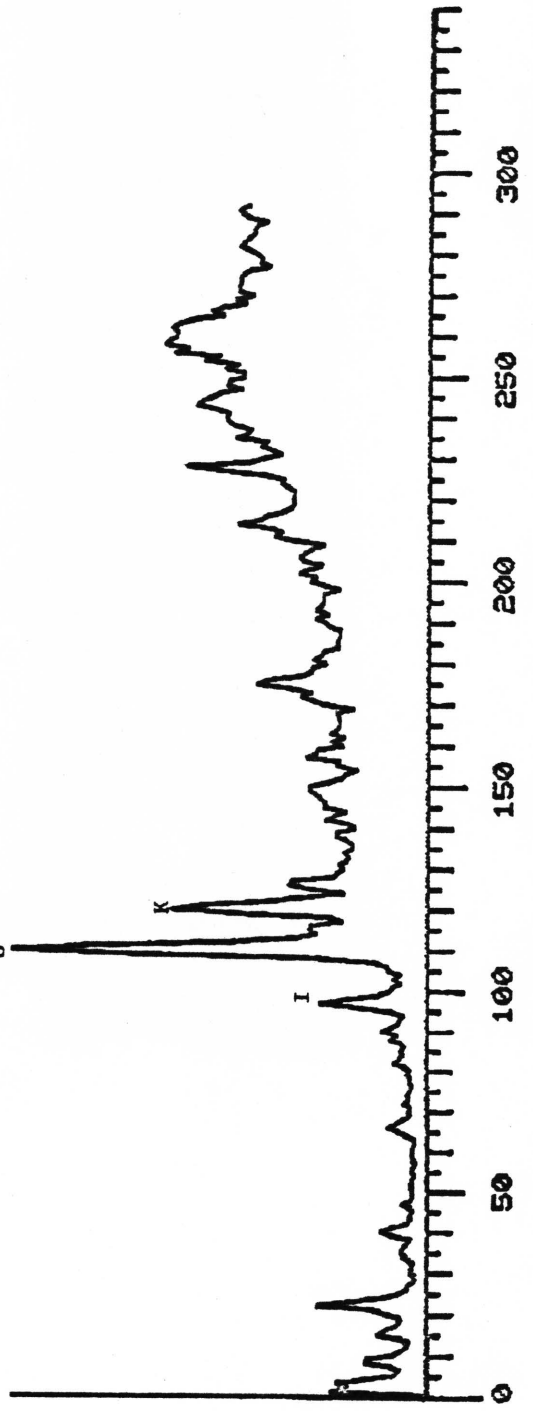

FIGURE 24 RECONSTRUCTED TOTAL ION GAS CHROMATOGRAM OF METHANOL ELUATE FRACTION.

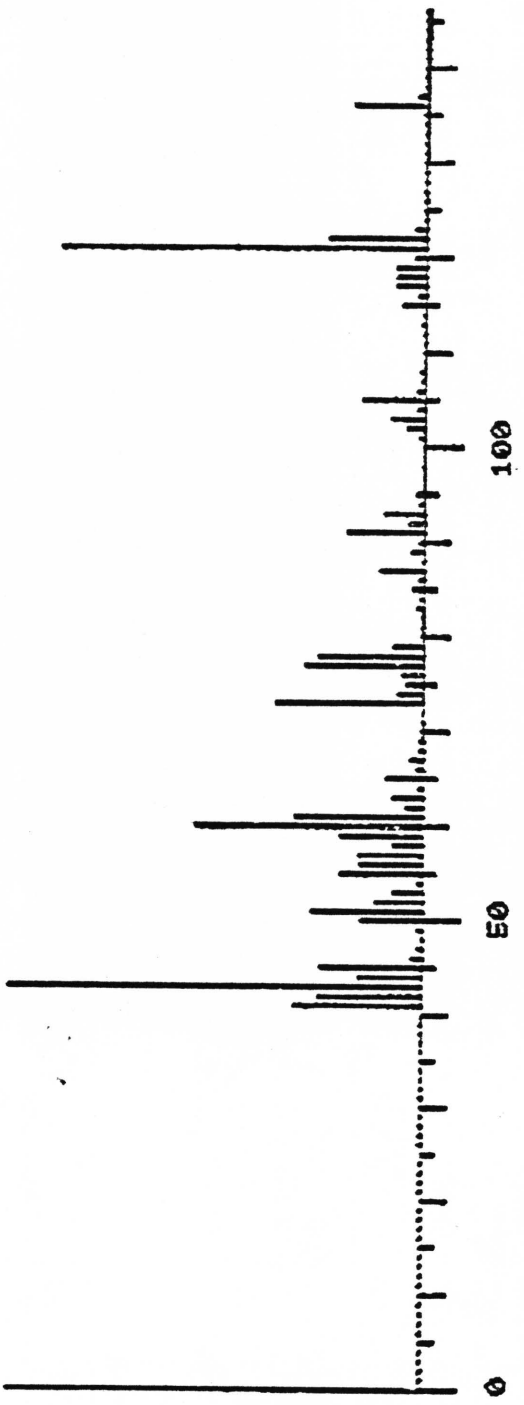

FIGURE 25. MASS SPECTRUM OF COMPONENT "J" OF FIG. 24.

FIGURE 26. MASS SPECTRUM OF COMPONENT "I" (ETHYL PHOSPHATE) OF FIG. 24.

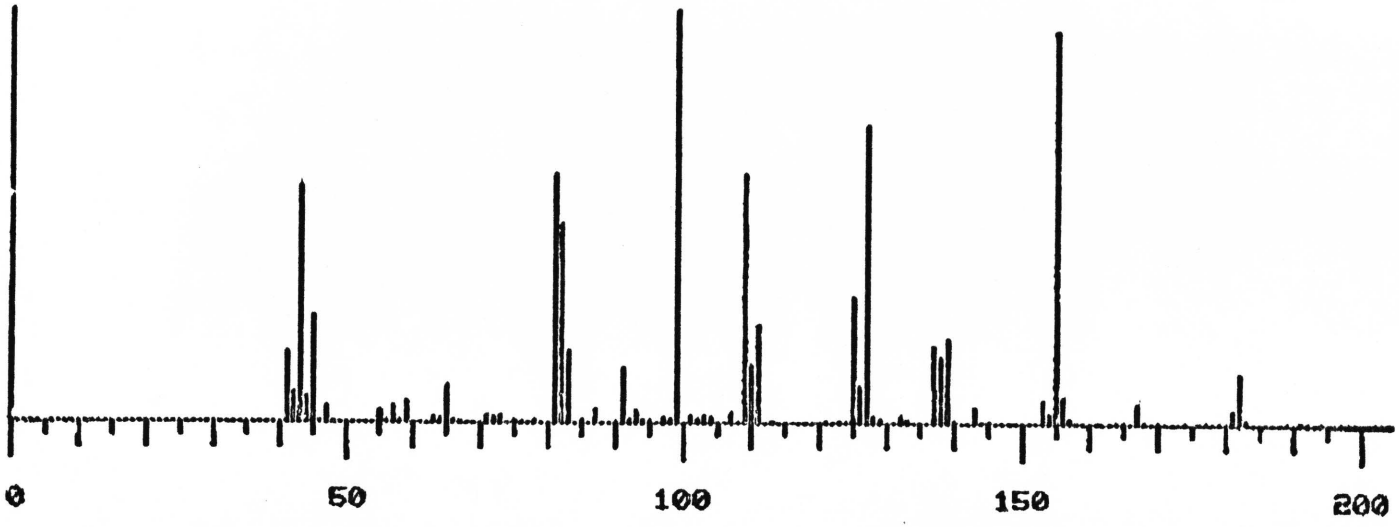

FIGURE 27. MASS SPECTRUM OF PHENOL FOUND AT SCAN 145 IN FIG. 24.

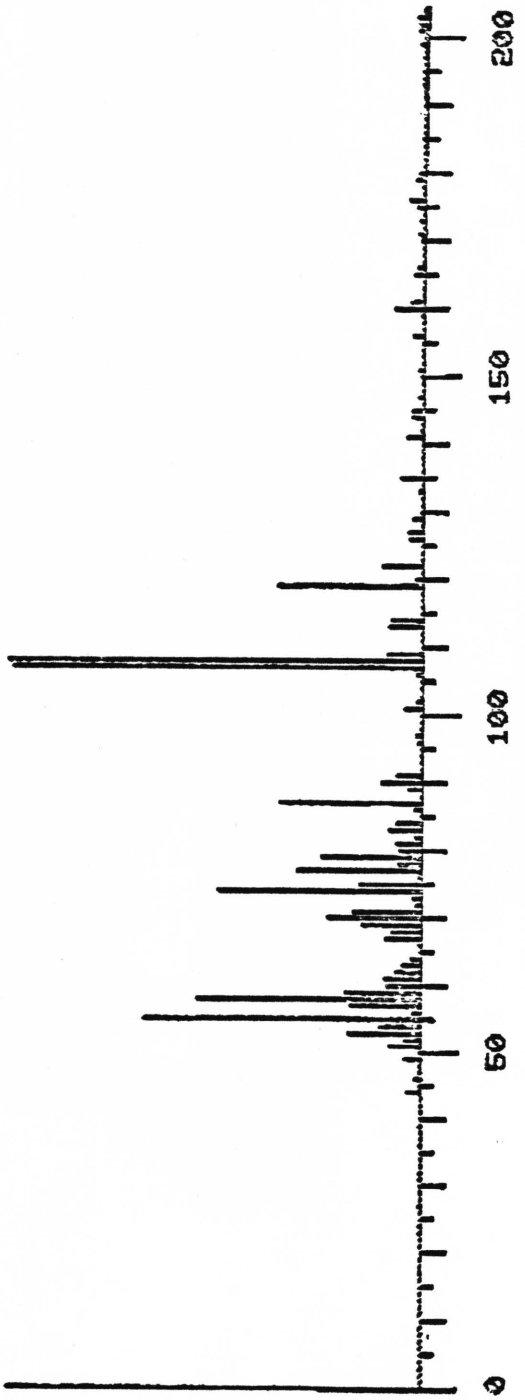

FIGURE 28. MASS SPECTRUM OF A CRESOL FOUND AT SCAN 156 IN FIG. 24.

C. Concentration of Selected Trace Metals in the MTBD

Introduction

This report summarizes the results of chemical studies on the heavy metals component of several batches of waste sludge from Shell Chemical Co., Deer Park, Texas. The metals chosen for analysis were Mn, Cu, Cr, Ni, Cd, Zn and Fe, based on preliminary reports which indicated that levels of some of these metals were much higher than in ordinary sediments or seawater. The project work was directed towards two main goals: (1) Accurately determine the concentrations of the metals in various batches of sludge; (2) Characterize the chemical form and behavior of the sludge heavy metals in seawater. This information is pertinent to understanding and predicting the transport and accumulation of heavy metals from such waste materials dumped in open ocean waters.

Methods and Materials

Samples of sludge material were obtained in either clean glass bottles or acid-washed Nalgene plastic bottles. The samples were subsequently kept in a refrigerator at 2 - 4 C for the duration of the study period. All glassware for trace metals work was washed in 50% HCl overnight.

Trace Metals Analysis

An appropriate volume of sludge (usually 10 ml) was measured out by graduated cylinder and placed in a porcelain Coors crucible. The sample was then combusted at 500 C in a muffle furnace overnight (ca 16 hr). The oven was at room temperature

when the sample was first placed in it; thus heating to 500 C was gradual. Similarly, combusted samples were allowed to cool slowly to room temperature before removing from the oven.

An initial experiment was performed to evaluate methods of oxidation of the organic material in the sludge. Dry ashing in the above manner was compared to wet digestion of sludge in a 3:1 mixture of nitric and perchloric acids at ca 100 C. Because little difference in the methods was observed at the levels of metals which were present in the sludge, the dry ashing technique was subsequently utilized.

The combusted material was dissolved in 3N HCl and warmed on a hot plate for a few minutes. The solution was next filtered through Whatman # 42 paper and brought to volume in a volumetric flask. This solution was then analyzed for Fe, Mn, Cu, Cr, Ni, Zn and Cd by flame atomic absorption spectrophotometry.

The instrument used was a Perkin-Elmer 303 equipped with a Recorder Readout and scale expansion and a 10 mv recorder. Matrix interference was evaluated for several elements (Cd, Ni and Zn) by using the standard additions technique and found not to present a problem. Background corrections for non-specific absorption were regularly obtained by using non-absorbing lines for each metal. The non-absorbing lines used were: Fe, 247.3 nm; Cu, 322.9 nm; Cr, 352.0 nm; Ni, 231.6 nm; Cd, 226.5 nm; and Zn, 220.2 nm.

The precision of the analytical technique appears quite good from the variation in replicate analyses. The percent deviation for each metal was Fe, 4%; Mn, 2%; Cu, 3%; Cr, 5%;

Ni, 8%; Cd, 8%; and Zn, 7%. The accuracy of the technique was verified by several analyses of a National Bureau of Standards sample, orchard leaves reference material # 1571. For a sample size of N.B.S. material corresponding to 10 ml of sludge (the regular amount used), the results for Fe, Mn, Cu, Cr, and Ni were all within 10% of the N.B.S. certified values. Only for Cd and Zn were greater variations found, and these ranged from 10 - 50% lower than expected values.

Isolation of Sludge Particulate Material

For separation of sludge into soluble and particulate phases, Whatman # 42 filter paper was used. This paper will retain very fine crystalline precipitates. After filtration of seawater suspensions of the sludge, filters with particulate material were combusted and the residue analyzed as described above. Filter blanks were run for all determinations.

Results

Sludge # 1

Heavy metals were measured on the settled solids fraction of sludge # 1, i.e., the denser material which settled out of the sample upon standing for several days. The concentrations are listed in Table 1 on the basis of µg per ml of wet solids. These data, therefore, represent a severalfold concentration of the metals over the initial sludge sample.

Sludge # 2

Concentrations of heavy metals in sludge # 2 are compared in

Table 1. Concentrations of heavy metals in Shell waste # 1. Values in µg/ml of liquid sludge ± S. D. for 4 determinations. Samples were digested in nitric + perchloric acid.

Metal	Batch # 1
Fe	99.4 ± 9.6
Mn	1.5 ± 0.2
Cu	2.4 ± 0.1
Cr	91.4 ± 5.4
Ni	1.6 ± 0.4
Cd	< 0.2
Zn	8.4 ± 1.0

Table 2 on the basis of µg per ml liquid sludge and µg per g dry wt suspended particulate material in the sludge.

Heavy metal concentrations in the particulate fraction of sludge # 2 are also presented in Table 2. Cadmium showed the most percentage in the soluble form (ca 15%), while essentially all of the other metals were retained in the particulate fraction.

Effects of washing the particulate material with various solvents can be interpreted from the data in Table 3. Seawater appeared to remove very little Cr, Zn, or Cd; at most, some 20% of the particulate-bound Ni was solubilized. Deionized water (comparable to fresh water) did solubilize about 50% of the Cd, in addition to 20% of the Ni.

Treatment with acetic acid-hydroxylamine reagent removed varying amounts of all metals from the particulate material (same table). Two groups of metals could be discerned: Cu, Cr, and Zn which were reduced 15 - 20% by this treatment; and Mn, Fe, Ni, and Cd which were desorbed some 50 - 60%.

Sludge # 3

The concentrations of heavy metals in sludge # 3 are expressed in Table 4 on the basis of µg metal per ml of liquid sludge and µg metal per g dry wt of suspended particulate material in the sludge. When the sludge was fractionated into soluble and particulate phases, the heavy metals showed the distribution indicated in the same table. Since all metals except Cd and Mn were predominantly (> 90%) in the particulate phase, the remainder of the studies were directed towards characterizing the nature of the association between

Table 2. Heavy metal concentrations in Shell waste # 2. Values in µg/ml of liquid sludge compared to µg/g dry wt particulate material in sludge. Data for particulate fractions given on µg/g dry wt basis. Values are ± standard deviation for 3 determinations.

Metal	Whole Sludge (µg/ml)	Whole Sludge (µg/g)	Particulate Fraction (µg/g)
Fe	132.0 ± 9.0	7432	7432
Mn	2.0 ± 0.2	113	113
Cu	1.9 ± 0.2	107	101
Cr	20.0 ± 5.5	1126	1126
Ni	2.5 ± 0.6	141	135
Cd	0.065 ± 0.01	3.66	2.82
Zn	9.0 ± 0.7	507	507

Table 3. Heavy metal concentrations (µg/ml sludge) in particulate fraction of Shell waste # 2, before and after various extraction procedures.

	Particulate Fraction			
Metal	Untreated	Deionized Water-Rinsed	Seawater-Rinsed	Hydroxylamine-Treated
Fe	132.0	--	--	56.0
Mn	2.0	--	--	0.6
Cu	1.8	--	--	1.5
Cr	20.0	18.0	18.0	16.0
Ni	2.4	2.0	2.0	1.2
Cd	0.05	0.02	0.05	0.02
Zn	9.0	8.5	9.0	8.0

Table 4. Total concentration of heavy metals in sludge # 3 and in particulate and soluble fractions. Values in µg/g dry wt or µg/ml liquid sludge ± standard deviation for at least 3 determinations.

Metal	Whole Sludge (µg/ml)	Whole Sludge (µg/g)	Particulate Fraction (µg/g)	Soluble Fraction (µg/g)
Fe	46.54	1820 ± 57	1540 ± 50	350 ± 8
Mn	0.61	24 ± 0.96	18 ± 0.8	6 ± 0.3
Cu	1.58	62 ± 8	58 ± 2.0	2 ± 0.2
Cr	22.27	808 ± 45	796 ± 27	< 10.0
Ni	1.41	56 ± 6	51 ± 6	6 ± 2.8
Cd	0.026	1.0 ± 0.2	< 0.8[a]	< 0.8[a]
Zn	4.40	172 ± 11	163 ± 5	10 ± 3.5

[a]Limit of detection was 0.8 µg Cd per g dry wt suspended material.

the particulate material and the metals.

The effect of diluting the sludge into various amounts of seawater and deionized water is apparent from examination of Table 5. Seawater solubilized a small portion of the Mn and Zn, while other metals were unaffected at their limits of detection. Deionized water, however, solubilized about 20% of the Ni in addition to Mn and Zn. These results hold over the seawater pH range from ca 6 to 8.0.

Two commonly-used chelating agents, ethylene dinitrilotetra-acetic acid (EDTA) and citric acid, were tested for their ability to remove metals at pH 7.8 from the sludge particulate material. Table 6 summarizes these data. Only EDTA at the highest concentration showed evidence of significant activity, chelating about 50% of the Fe and a slight amount of Ni and Mn. Cr, Cu, and Zn appeared totally unaffected.

The particulate fraction was found capable of adsorbing additional metals out of seawater. This scavenging ability was demonstrated by spiking seawater-sludge mixtures with known amounts of pure metal compounds. The data in Table 7 show that extra Cu and Cr were readily and completely adsorbed to the particulate fraction. Manganese at the concentration occurring naturally in Gulf of Mexico bottom water (in this sample 53 µg/l) was only partially adsorbed. The results with Ni are particularly interesting; Ni was poorly adsorbed when added by itself and even this small amount of adsorption was negated when extra Cu and Cr were also present. Hence, the particulate material in sludge # 3 appeared to complex or adsorb metals selectively.

Table 5. Effect of diluting sludge # 3, with sea water and deionized water. Suspensions were made by mixing 10 ml sludge with an appropriate amount of seawater or deionized water for 18 - 24 hrs. Particulate fraction was collected by filtering through Whatman # 42 paper and trace metals measured on the suspended material. Values are µg per g dry wt ± standard deviation for at least 3 replicates.

Metal	Sludge Particulate Fraction Untreated	10 ml Sludge + 40 ml Seawater	10 ml Sludge + 90 ml Seawater	10 ml Sludge + 190 ml Seawater	10 ml Sludge + 90 ml Deionized Water
Fe	1540	1580 ± 83	1560 ± 60	1530 ± 42	1560 ± 73
Mn	18	11 ± 1.9	13 ± 1.4	10 ± 1.5	11 ± 1.4
Cu	58	60 ± 3	56 ± 1.0	61 ± 5	58 ± 4
Cr	796	770 ± 42	785 ± 12	793 ± 50	773 ± 28
Ni	51	50 ± 8.0	47 ± 1.4	50 ± 2.5	40 ± 3
Cd	< 0.8	< 0.8	< 0.8	< 0.8	< 0.8
Zn	163	140 ± 21	142 ± 32	137 ± 28	140 ± 35

Table 6. Effect of treating sludge # 3 with organic chelating agents in seawater. Values are µg/g dry wt ± standard deviation for 3 determinations.

Metal	Particulate Fraction After Exposure to 40 ml Seawater	Treated with 10^{-3} M Citric Acid	Treated with 10^{-5} M EDTA	Treated with 10^{-3} M EDTA
Fe	1600 ± 25	1600 ± 22	1590 ± 10	1010 ± 51
Mn	15 ± 0.7	16 ± 1.4	14 ± 0.7	11 ± 1.4
Cu	63 ± 3	66 ± 0.7	65 ± 4.8	67 ± 10
Cr	780 ± 55	738 ± 31	805 ± 48	790 ± 42
Ni	54 ± 2.8	56 ± 1.4	48 ± 2.8	45 ± 1.4
Cd	< 0.8	< 0.8	< 0.8	< 0.8
Zn	153 ± 2.8	147 ± 21	158 ± 12	146 ± 18

Table 7. Adsorption of extra metals added in seawater solution to sludge # 3 suspended matter. Single metal means an extra amount of only that metal was added. Combined metals means the following mixture was added to 10 ml sludge: 10 µg Cu, 10 µg Ni, 200 µg Cr, and 0.1 µg Cd. Values are µg/g dry wt ± standard deviation for 3 determinations.

Metal	Sludge Particulate Fraction After Exposure to 40 ml Seawater	Sludge Particulate Fraction After Exposure to Extra Metals		% Adsorption of Added Metal	
		Single Metal	Combined Metals	Single Metal	Combined Metals
Fe	1580				
Mn	11	32 ± 1.4		57	
Cu	60	100 ± 3.5	82 ± 5	100	82
Cr	770	1470 ± 10	1440 ± 10	100	97
Ni	50	57 ± 2	48 ± 4	15	0
Cd	< 0.8	1.2 ± 0.2	0.9 ± 0.1	100	50
Zn	140				

Discussion

Adsorption of heavy metals to suspended particulate material in seawater is not unexpected (Helz et al., 1975; Windom, 1976; Trefrey and Presley, 1977). The interesting observation is that the metals are so tightly bound to the particulate fraction of these sludge samples. The acid-hydroxylamine reagent was found by Chester and Hughes (1967) to leach from clay minerals trace metals which are not part of the lattice structure of the mineral. Ferro-manganese nodules were almost completely dissolved by this reagent. Hence, the results from the hydroxylamine leach of sludge # 2 suggest that either a considerable portion of the Cu, Cr, Zn, and Ni are in lattice positions of a mineral or that these metals are part of a particulate organo-metallic complex.

Since the particulate material was readily dissolved by hot nitric acid (wet digestion) and almost completely combusted by dry ashing at 550 C, very little sand or clay mineral sediment particles appear to be present. The fairly high organic carbon content (around 16% for sludge # 2 and # 3) makes it more likely that the particulate material is precipitated organic material, possibly polymeric in nature, with a high metal-complexing capacity. This would also correlate with the apparent selectivity of the material for adsorbing metals.

Heavy metal concentrations in sludge # 3 suspended material were compared to those for suspended particulate material in natural seawater samples (Table 8). By comparing Fe and Mn values, it is apparent that the sludge particulate material is highly enriched in Cu, Cr, Zn, Ni, and probably Cd. Although Fe

Table 8. Comparison of heavy metals[a] in naturally-occurring suspended material with concentrations measured in particulate fraction (= susp. matter) from sludge # 3.

Metal	Miss. River Suspended Matter[b]	Nearshore Gulf Susp. Matter[b]	STOCS Gulf of Mexico Susp. Particulates[c]	Sludge # 3 Particulate Fraction
Fe	47,400	50,200	15,000	1,540
Mn	1,307	1,191	567	18
Cu	42.3	55.6	78	58
Cr	72.5	72.8	48	796
Ni	55.6	58.5	58	51
Cd	1.4	2.0	5.4	< 0.8
Zn	184	226	300	163

[a] All values are µg/g dry wt.

[b] From Trefrey and Presley (1977).

[c] Average values, from Barnes (1976).

and Mn in the sludge are only 5 - 10% of the levels found in natural suspended matter, the other metals are at the same level or, in the case of Cr, ten times higher.

The impact of adding this metal-laden suspended material to the open ocean can be illustrated in the following manner. Normal concentrations of suspended particulate material fall in the range of 5 - 10 mg/l for offshore waters (Spencer and Sachs, 1970; Barnes, 1976). If the sludge were diluted immediately upon dumping to 0.1% of its full strength, the concentration of suspended particulate material would be 0.001 x 25,000 = 25 mg/l. This means that seawater in the immediate area of dumping would then have an increased load (by a factor of 3) of suspended material which is itself enriched in Cr, Cu, and Zn. Continued dumping of the sludge would offset the detoxifying effect of dilution by seawater, since the particulate material would gradually build up around the dumpsite.

The results of mixing sludge with seawater can be extrapolated to the natural ocean situation with a high degree of relevance. Many of the laboratory tolerance studies where biological effects of the sludge are determined have utilized seawater dilutions of the sludge comparable to those listed in Table 5 (5 - 20%). Since seawater desorbed very little of the potentially toxic metals present at high levels (viz. Cu, Cr, Zn, and Ni), heavy metal effects on organisms of sludge mixed in seawater could result from exposure to or ingestion of particulate material.

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Report for NOAA on Ocean Dumping in the Gulf of Mexico

Effects of Wastes on Growth of Microalgae

by

C. Van Baalen, J. Batterton

Introduction

The purpose of this work was to assay samples of waste material for inhibition of growth of algae. Three samples (noted as I, II, and II) supplied to us were tested for toxicity to six microalgae. The test organisms, two blue-green algae, two green algae, and two diatoms represent three major divisions of algae.

Methods

Samples of waste material were frozen upon arrival and stored at -10°C. Just before testing, frozen aliquots of each sample were thawed and autoclaved (121°C, 15 min).

The microalgae were grown in the synthetic sea water medium ASP-2 (Provasoli, McLaughlin and Droop 1957; Van Baalen 1962), using the test-tube culture technique of Myers (1950). Green algae used were Chlorella autotrophica, strain 580 and Dunaliella tertiolecta, strain DUN (both obtained from R. R. L. Guillard); the blue-green algae used were Agmenellum quadruplicatum, strain PR-6, and Coccochloris elabens, strain 17a (isolates of this lab); and the diatoms were Cylindrotheca sp., strain N-1, and Chaetocerous simplex (isolates of this lab). All cultures were pure except C. simplex. C. simplex cultures were incubated at 27°C, all others at 30°C. The cultures were illuminated continuously; C. simplex with F20T12-D fluorescent lamps and the others with F40CWX fluorescent lamps. All cultures were continuously aerated with a 1.0 ± 0.1% CO_2-in-air mixture. Growth was estimated turbidimetrically using a Lumetron colorimeter Model 402-E with a red glass filter (660 mm). For

simplicity the data is reported in generations per day.

Autoclaved waste material was added directly to sterile growth medium. No additions were made to control cultures. Duplicate cultures were used in all assays. The culture tubes were inoculated with $\sim 10^5$ cells/ml and incubated immediately. Occasionally sample turbidity made optical growth measurements difficult, thus chlorophyll a analyses (90% redistilled acetone extracts) were done to aid in interpretation of growth data. An outline of the liquid culture method is given in Fig. 1. Algal lawn assays were done according to the method described previously (Pulich et al. 1974).

Results

Sample I was tested in two ways, as a whole sample and as its liquid phase and solid residue obtained by settling (18 hrs).

The whole sample was checked by placing material absorbed on a washed filter paper disc (S & S No. 740E, 12.7 mm) onto an algal lawn, $\sim 10^5$ cells/ml, in agarized medium. The petri dishes were incubated under growth conditions for 4 - 6 days. There was no inhibition of growth (clear zones surrounding the disc) with organisms PR-6, 580, or N-1 (data not shown).

However, in liquid culture assays whole Sample I somewhat inhibited growth of C. simplex (Table 1). The toxicity of Sample I was associated with the residue (material obtained after settling) but the amount required was rather high. The liquid phase was not toxic even at 50% (v/v) (Table 1).

Table 2 summarizes the growth rate data in liquid culture

Figure 1. Protocol of testing waste material samples

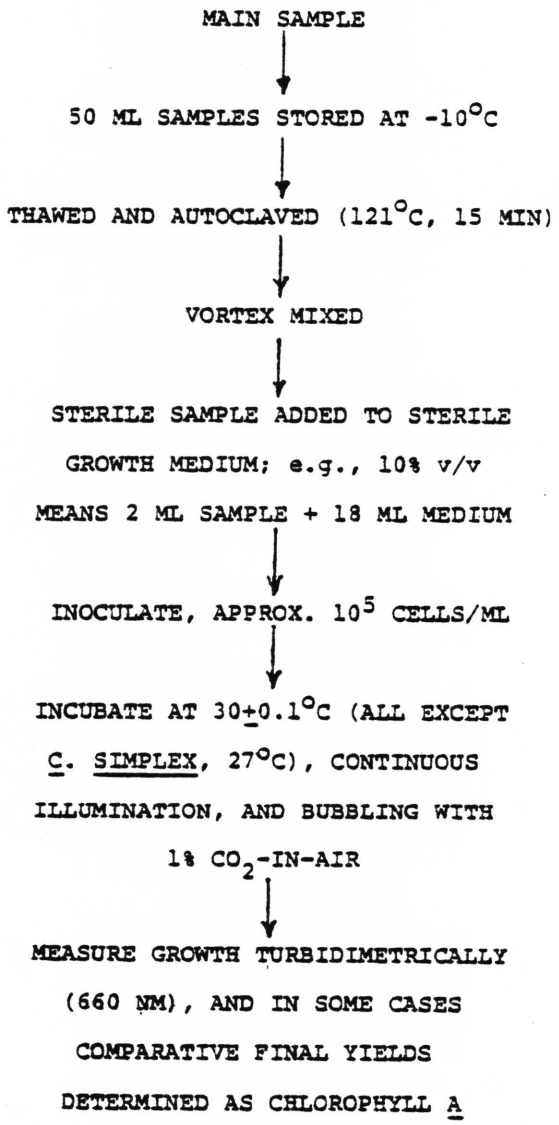

Table 1. Effect of whole Sample I and residue on growth of *Chaetocerous simplex*.

Type of Addition to Medium (20 ml)	Generations/Day	Lag Time in Initiation of Growth (h)
Whole sample		
control (disc only)	5.8±0.2	0
disc + absorbed sample[1]	4.4	17
Residue, mg wet wt/disc		
0	6.0±0.2	0
1.5	5.6	0
3.0	5.1	2
7.0	4.4	16
∿20	NG-7[2]	--
Liquid phase, % v/v		
25	5.9	0
50	5.9	0

[1]Filter paper disc dipped into Sample I, then added to test culture

[2]NG-7 means no growth after 7 days incubation

Table 2. Effect of autoclaved Samples II and III added to the medium (v/v) on growth rates of algae.[1]

	SAMPLE II			SAMPLE III		
ALGAE	0	5%	10%	0	5%	10%
Blue-greens						
PR6	5.0±0.4	3.0	2.0	5.1±0.4	3.6	1.6
17a	3.6±0.4	2.7	1.9	3.9±0.4	2.6	2.2
Greens						
580	2.6±0.3	1.9	1.2	2.2±0.3	2.9	2.5
Dun	2.6±0.3	1.9	1.6	2.4±0.3	2.4	2.8
Diatoms						
N-1	4.1±0.3	NG-2[2]	NG-2	4.2±0.3	3.3	2.9
C. simplex	5.1±0.5	NG-2	NG-2	5.1±0.5	NG-7	NG-7

[1]Growth rates expressed as generations/day, at 30° (*C. simplex*, 27°) under continuous illumination and aeration with 1% CO_2-in-air

[2]NG-2 or -7 means no growth in 2 or 7 days after inoculation

of the six test algae with Samples II and III. Samples II and III were toxic to the blue-green algae, growth rate decreased about one-half at the highest concentration tested (10% v/v). This pattern of response was also seen with the two green algae and Sample II. Sample III showed little toxicity to the green algae, indeed for reasons unknown, some stimulation of growth was seen.

Of the algae tested, the diatoms were the most inhibited by both samples. Sample II completely suppressed growth of both species. Sample III caused only a partial reduction in growth rate of N-1 at 10% (v/v), but no growth was obtained with C. simplex even after 7 days incubation. Further experiments with C. simplex and Sample III showed growth (4.2 generations/day) would occur at a concentration of 0.5% (v/v) but not at 2.5% (v/v).

Conclusions

The results of this study have shown that each of the samples were toxic to at least some of the algae tested. Samples I, II and III were especially toxic to the diatoms. It is of particular interest that Chaetocerous simplex emerged as the most sensitive test alga. Possibly this reflects a greater sensitivity (less metabolic versatility?) of offshore diatoms to pollution. In the case of Sample I the toxicity was associated with the solid phase.

Taken as a whole, the data on the microalgae suggest that caution is advisable if promiscuous dumping of samples like

those assayed herein is allowed. Microscopically, the samples appeared to have high bacterial counts, their original organics were thus probably well worked over, yet the samples still evidenced toxicity. In this connection it is of interest that in Sample I the toxicity was associated with the sediment fraction, which could have a long environmental half-life. Short-term toxicity effects, such as selection or enrichment of certain species in the phytoplankton are also predictable (Table 2) with perhaps deleterious effects through the food chain.

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Report for NOAA on Ocean Dumping in the Gulf of Mexico

Toxicity of Biosludge on Marine Invertebrates

by

J. A. C. Nicol

Introduction

Our previous reserach on the toxicity of industrial products to marine invertebrates dealt with petroleum oils (Lee and Nicol, 1977; Lee et al., 1977). We found deleterious action in survival, growth rate, feeding, respiration, behavior and fecundity. From our experiments with these techniques, we began a study of the effects of biosludge, destined for ocean dumping, on two marine invertebrates. For the first year we concentrated on survival, growth and reproduction of a benthic animal (an amphipod) and a pelagic one (a sergestid shrimp).

Materials and Methods

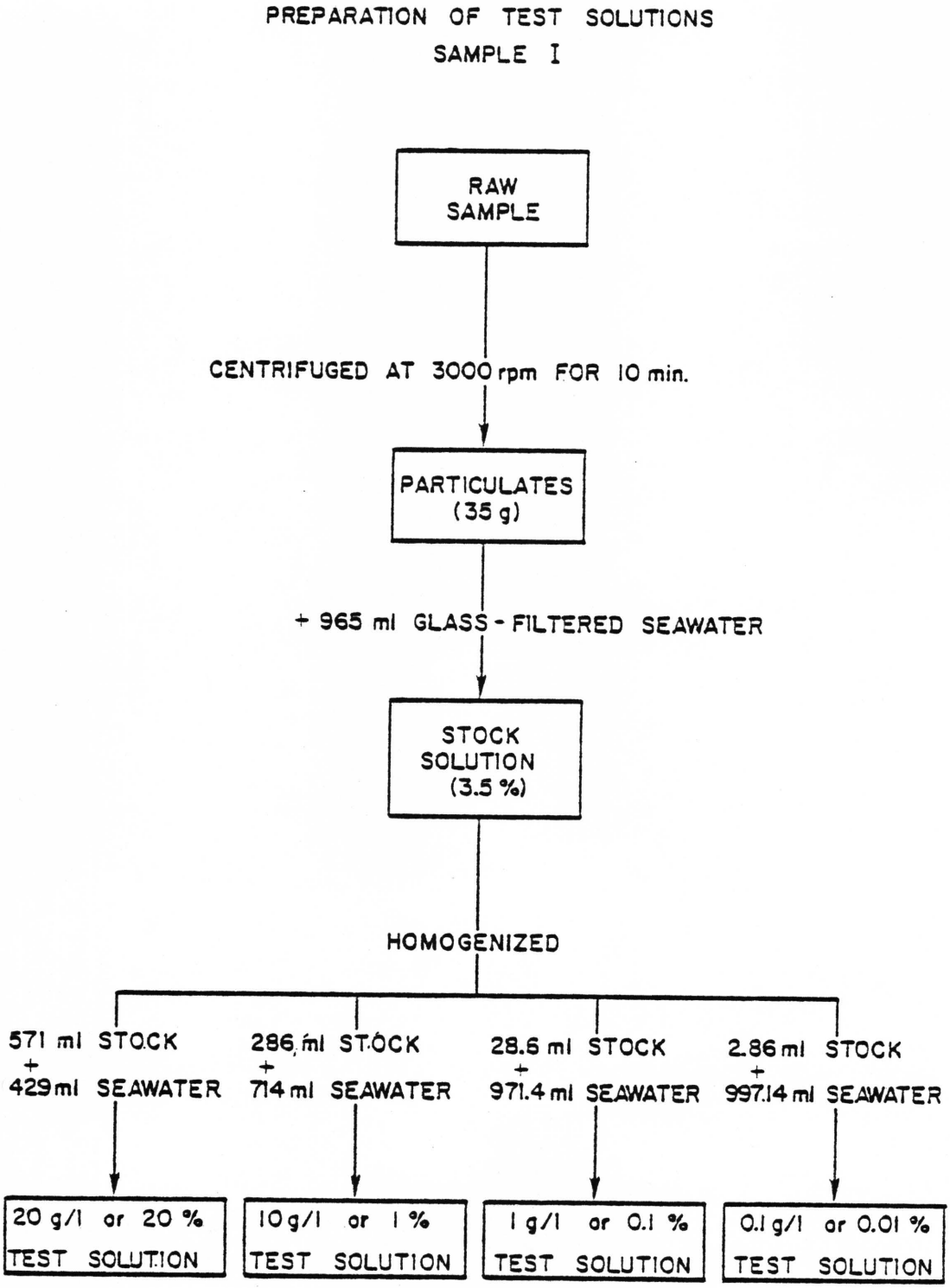
Four lots of biosludge were supplied in barrels by the Shell. They are designated Sample I, II, III and IV.

Sample I contained much solid material and was filtered by the Chemistry Department of the Port Aransas Laboratory; the solid material recovered by filtration was tested. It was centrifuged, and the material in the pellet was suspended in sea water, 35g in 965 ml of sea water. The suspension was homogenized in a Waring blender, and subsequently stirred to maintain the suspension. From this suspension, as stock solution, dilutions were made (see Figure 1, flow sheet).

Sample II contained much finer solid material. It remained fairly homogeneous. It was shaken vigorously before use, and aliquots were taken as required for testing.

Samples III and IV were fairly homogeneous, resembling

Figure 1. Flow chart for the preparation of the test solution.



Sample II. They were left undisturbed for 2 weeks, whereupon solid material settled, and the clear supernatant was siphoned off for use.

Offshore sea water was used as medium for the animals. It was passed through glass fiber filters, antibiotics were added, namely, penicillin G and streptomycin sulphate at 50 mg and 25 mg l^{-1}.

The test animals were Amphithoe valida and Lucifer faxoni. They were kept in culture bowls, 19 cm diameter, containing 1.5 l of sea water 30 o/oo. Bowls were covered with PVC film (Saran wrap), and were gently aerated. Amphipods were fed tropical fish food flakes and dried Ulva; shrimp were fed a mixture of rotifers and newly hatched Artemia. Temperatures were 24 $\pm$ 2° C.

Both LC_{50} and TL_{50} values were calculated by the method of Litchfield and Wilcoxon (1946).

Results

Sample I

Several concentrations (0.01 to 3%) were tested on the amphipod Amphithoe valida. Time to kill 50% of the test population for a 2.0% (20 l^{-1}) and 1.0% (10 l^{-1}) mixtures were 24 h and 33 h, respectively (Fig. 2). A later test showed that at the concentration of 2.0% the T_{50} was delayed to 48 h and there was no mortality at all for the population tested at 1.0% during 4 days' exposure (Fig. 3). The difference in results was probably caused by the different age groups used

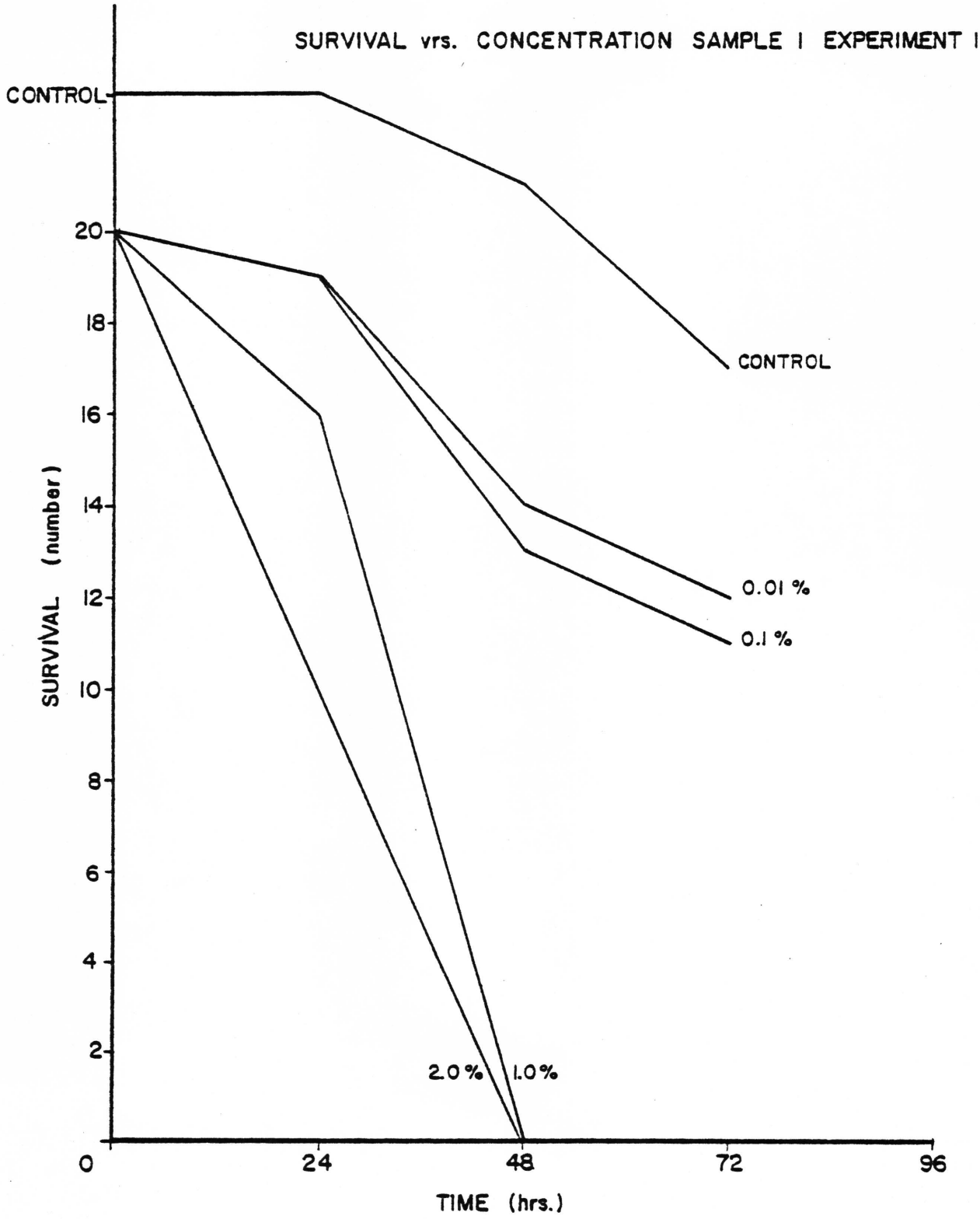


Figure 2. The survival of Amphithoe valida (10 weeks-old) at various concentrations of biosludge. Mixtures were made from the solid part of biosludge.

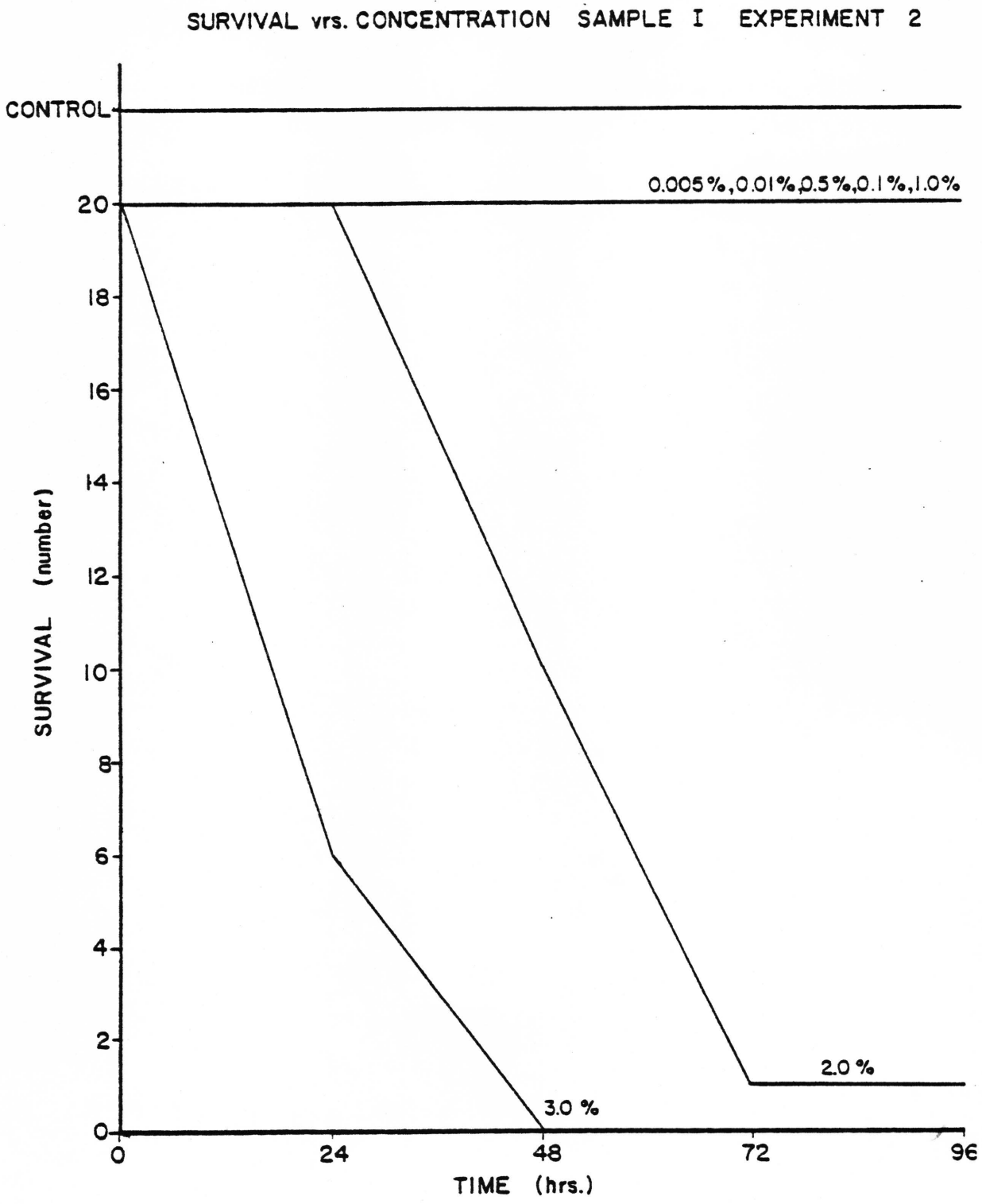


Figure 3. The survival of Amphithoe valida (6 weeks-old) at various concentrations of biosludge. Mixtures were made from the solid part of biosludge.

in the two series of toxicity test. Amphipods 10 weeks old were used in the first experiment and 6 weeks old in the later test.

Moults also were recorded. There was no difference between control and experiments in test solutions at concentrations < 0.01%. There were few moults in the 0.1% test solution (Fig. 4).

Sample II

For this experiment, the relative sensitivity of different age-groups of amphipods to the whole biosludge was determined. Age-groups used in this experiment were 3, 5 and 9 weeks old. Concentrations of biosludge tested varied from 4 to 10% (v/v) and exposure lasted for 96 h. We found that amphipods in either young or old stages were more sensitive than the just matured groups (5 weeks old). For example, in a 10% mixture, more than 90% of the test animals of age 5 weeks was still alive after 4 days' exposure, while for the other two ages, under the same conditions, the mortality was > 35%.

Sample III

In these experiments with amphipods the supernatant of biosludge was first autoclaved and then used in the test. Concentrations used were 10%, 20%, 30% and 40%. Exposure time was extended to 8 days. Mortality was checked every day. No mortality was observed in the 10% group. For the remaining groups (20%, 30% and 40%) the time to kill 50% of the test population was found to decrease with the concentrations and, in order, were 165 h, 105 h, and 81 h, respectively (Fig. 5).

The planktonic shrimp, Lucifer faxoni, was tested with

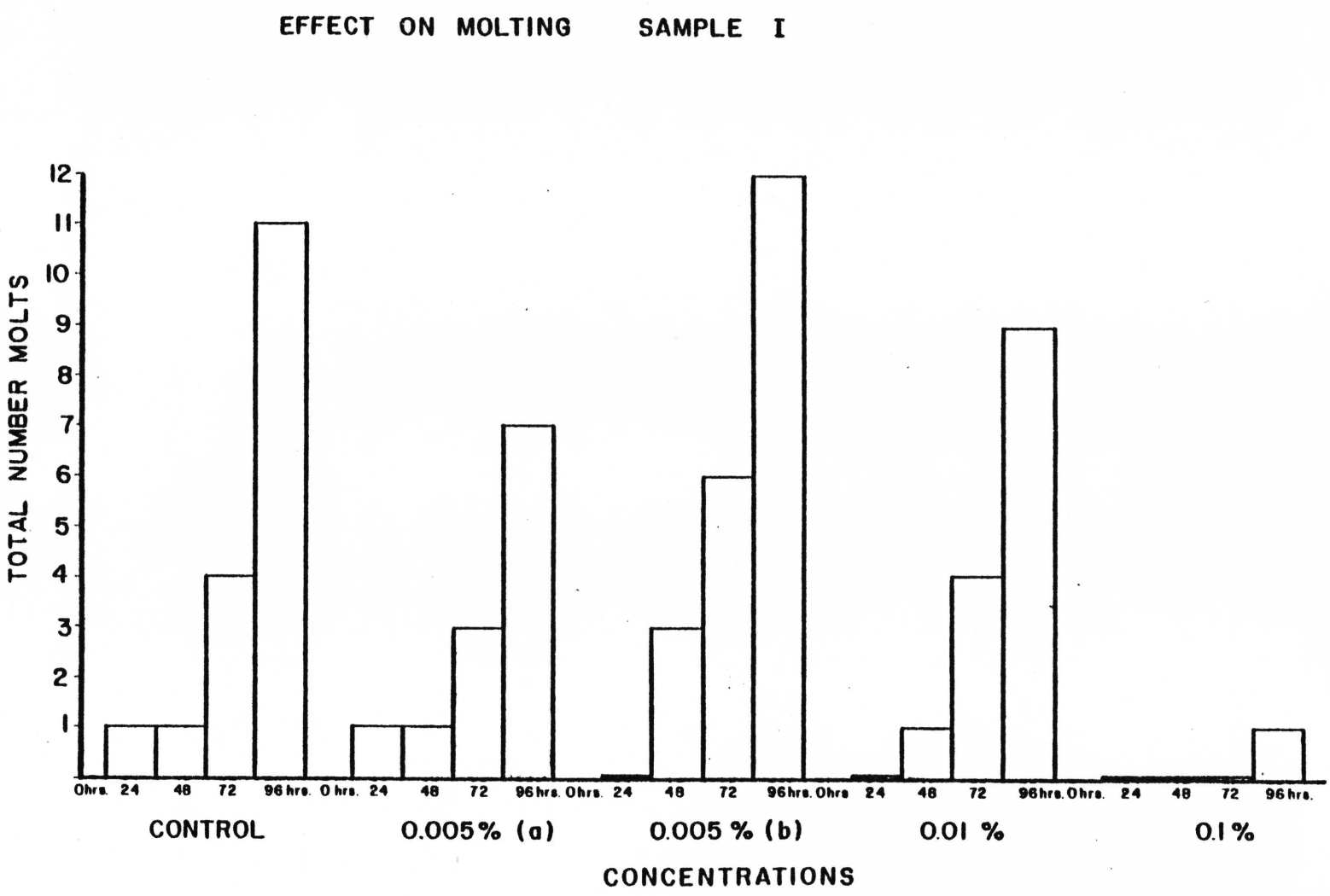


Figure 4. Effect of biosludge on the molting of Amphithoe valida (6 weeks-old). Mixtures were made from the solid part of biosludge.

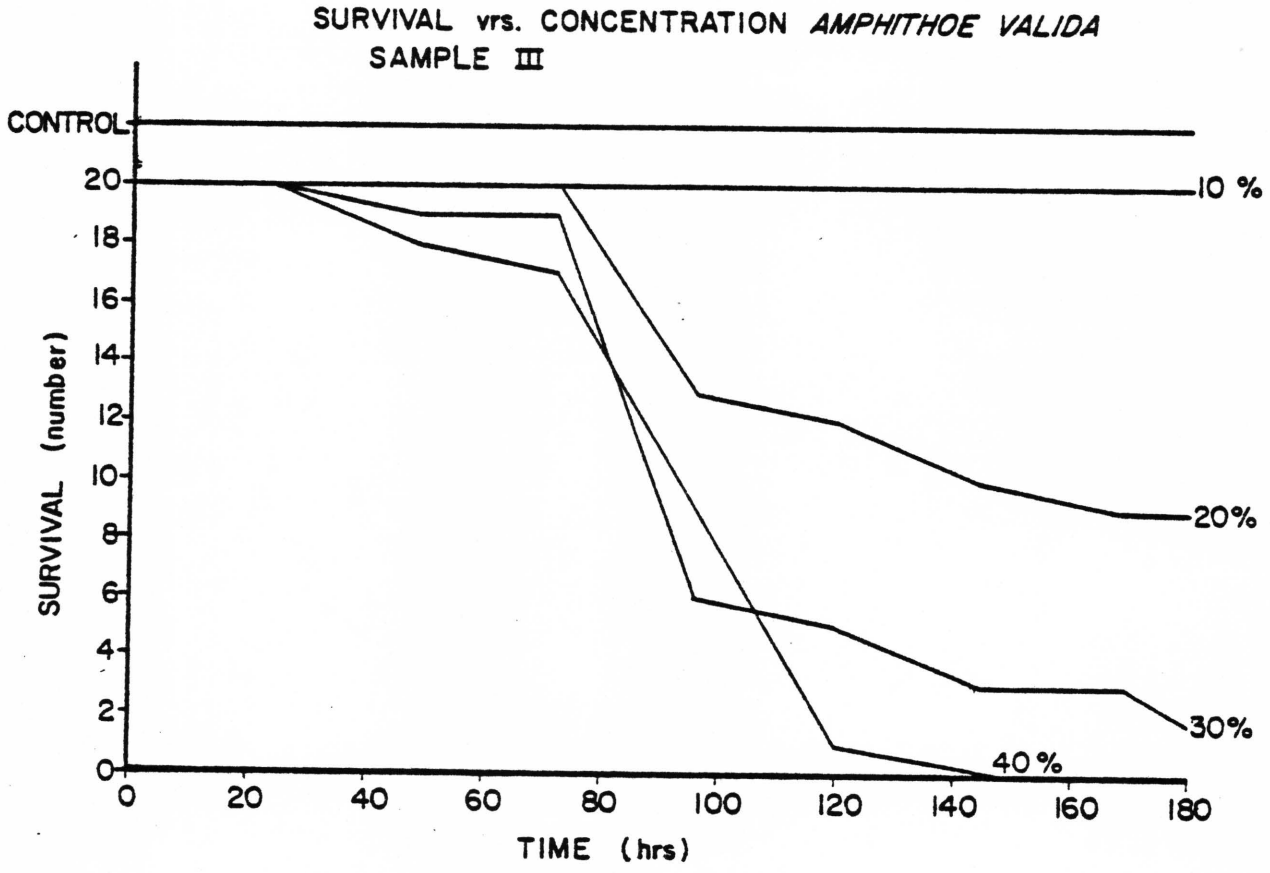


Figure 5. The survival of Amphithoe valida (3 weeks-old) in the supernatant of biosludge. Supernatant was autoclaved before animals were introduced.

autoclaved and non-autoclaved supernatant. The non-autoclaved solution was toxic at 10%, and all animals were dead in the 15% solution at 48 h (Fig. 6). The latter result was somewhat vitiated because of heavy bacterial growth. The autoclaved solution, even at 25%, was non-toxic at 180 h.

Sample IV

The data (in Fig. 4) for molting indicated that adverse effects of biosludge on amphipods may be found at much lower concentrations than that of 24 h or 96 h- LC_{50}. To verify this, experiments were carried out under conditions of low concentrations (< 10%) and a long period of exposure.

Four-weeks-old amphipods were introduced into the following concentrations of biosludge supernatant, 1%, 2%, 3%, 6%, 7% and 8%. A control was also provided as a check. At each concentration, 20 individuals, with a male to female ratio of about 1.0, were added. The test medium was changed once a week; at the same time, the total number of young released within that week was also determined for each concentration. The exposure lasted 2 months.

Results from this part of the experiment are shown in Fig. 7. Two significant patterns could be recognized. The reproductive potential for those groups exposed to concentrations > 6% was significantly depressed as compared to that of control. Yet the amphipods which were exposed to concentrations < 3%, released more young than did the control group. The largest number (157) of young produced was recorded at the 3%

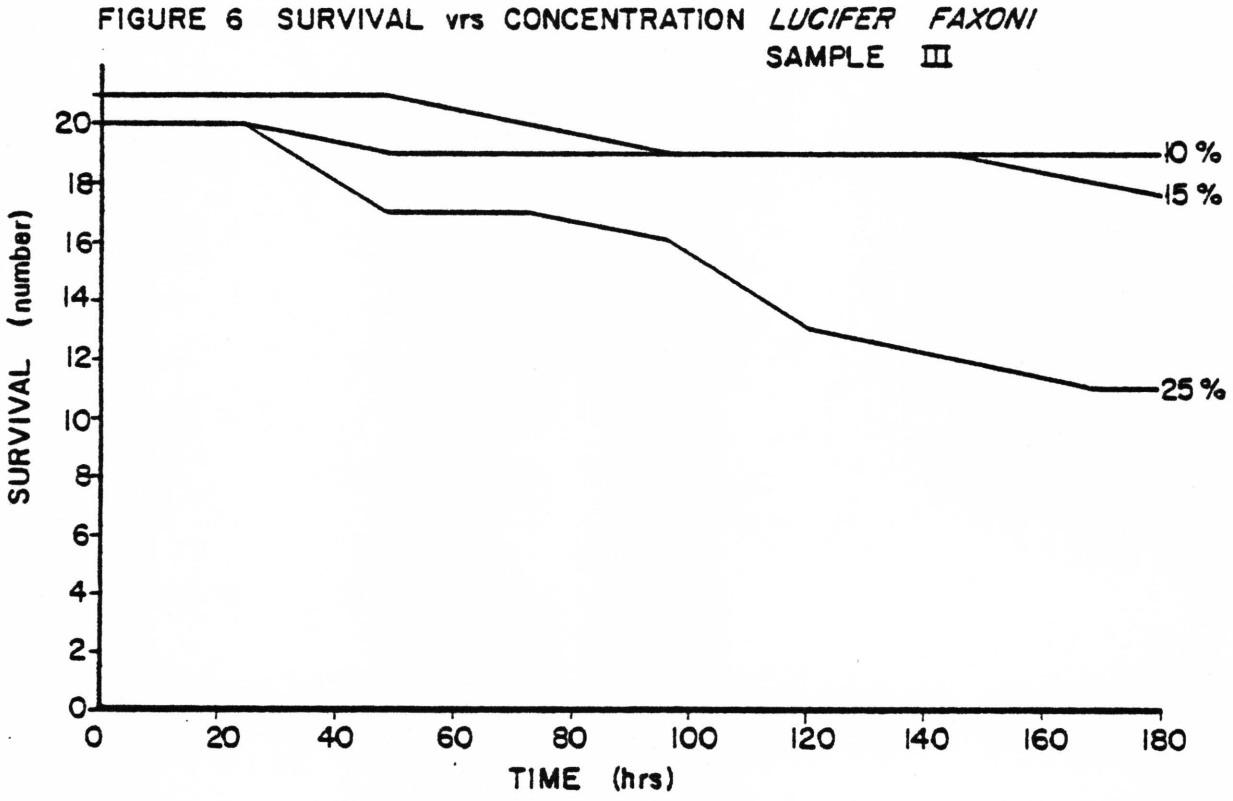


Figure 6. The survival of adult Lucifer faxoni in the supernatant of biosludge. Supernatant was autoclaved.

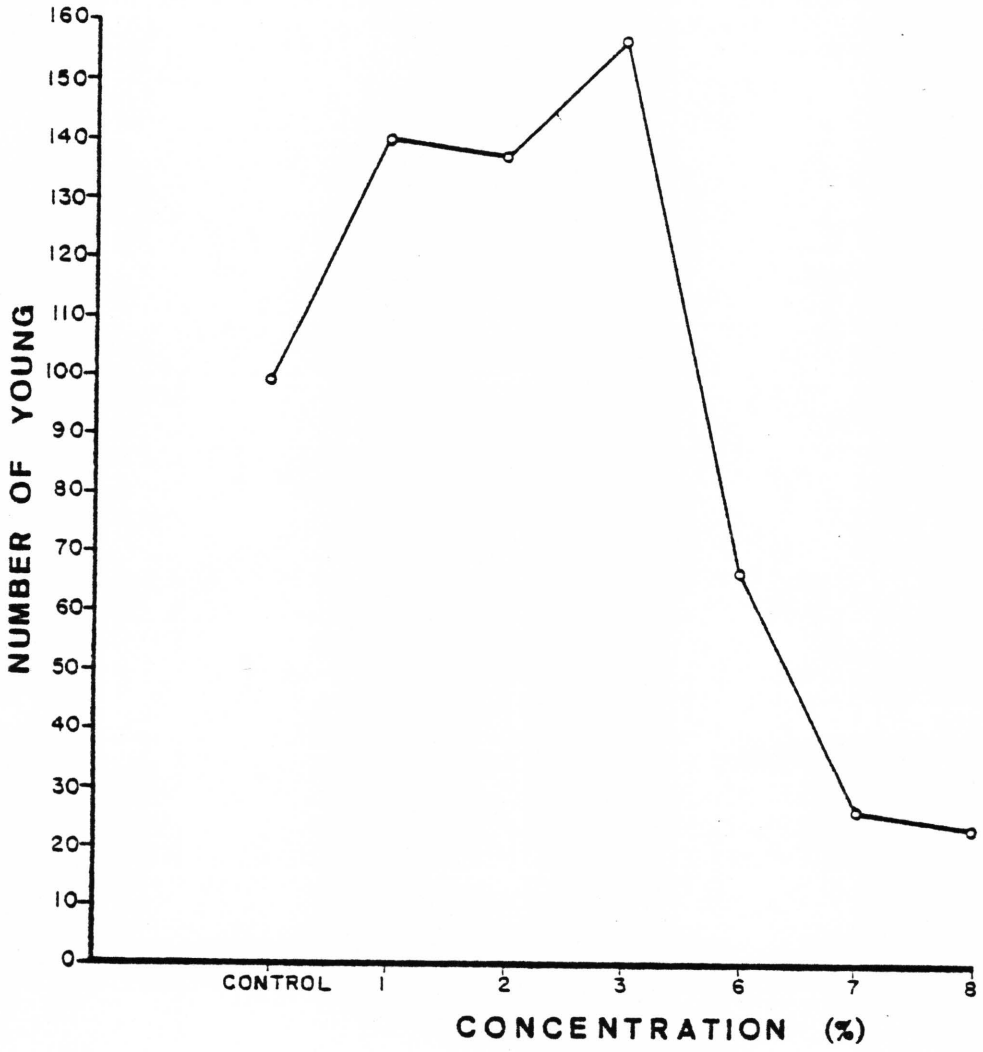


Figure 7. Effect of biosludge on the fecundity of Amphithoe valida (4 weeks-old). Biosludge was filtered through glasswool before used.

concentration and the least number (23) at the 8%. The total number of young released in the control was 99.

Two other experiments have also been carried out in conjunction with Dr. W. Pulich, who measured the heavy metal uptake of animals from the biosludge. Two species of marine crustaceans, Amphithoe valida, and Palaemonetes pugio (grass shrimp), were exposed to whole biosludge for 16 hours, in concentrations varying from 5 to 20%. At the end of the experiment, animals were rinsed with demineralized water several times and then passed to Dr. W. Pulich. Preliminary results showed significant changes in the total copper and iron of the treated group. Details were reported and discussed by Dr. W. Pulich.

Discussion and Conclusion

There was considerable difference between toxicities of the several samples of biosludge, perhaps because of the actual difference in the composition between samples or because of differences in methods of preparation (Siegel and Rader, 1974). For the amphipod, A. valida, the whole biosludge was acutely toxic at about 10% and the level of toxicity varied with the age. The supernatant from the suspension was also toxic, as shown by the responses of many marine invertebrates to pollutants (Roesijadi, et al., 1976; Rossi and Anderson, 1976), at a level of about 20%. These levels are for mortalities significantly greater than those of controls. Results for the planktonic shrimp were complicated by bacterial growth. When

autoclaved, the supernatant was much less toxic to both the amphipods and the shrimps. This procedure controls bacterial growth, but also affects the chemical composition of biosludge.

Chronic exposure of amphipods to low concentrations, < 10%, resulted in changes in both molting and fecundity. The changes in molting suggest that growth may be interfered at a concentration as low as 0.1%. However, data on reproduction showed that within the sublethal levels of biosludge, more young were released at lower concentrations than at higher concentrations, when compared to the control. This pattern was not unusual since a similar pattern has been observed on the respiration of L. faxoni when exposed to the water soluble fractions (WSF) of a No. 2 fuel oil. In Lucifer, the respiration rate rose with increasing concentration of WSF up to 30%, then fell as the concentration increased (Lee et al., 1978).

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Sensitivity of Open Gulf Fishes to Ocean Dumping Wastes

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Introduction

The purpose of this study is to determine whether respiratory metabolic responses of a marine fish are sufficiently sensitive at sublethal pollutant levels to suggest possible biological monitoring techniques.

The rationale of using respiratory scope -- the difference between oxygen consumptive rates at maximum sustained aerobic activity and at the maintenance or standard level -- for the assessment of environmental quality was suggested by Fry 1947 and elaborated in general physiological terms by Fry (1957, 1971). Since theoretical and empirical studies suggested that metabolic scope tends to be reduced under stressed systems, much research has been related to various environmental circumstances. Brett (1958, 1964, 1965, 1971), Brett and Glass (1973), and Brett *et al.* (1969) have shown that at optimal temperatures, scope and swimming performances are also related to optima in rations, assimilation, growth and related functions.

It is important to note that most fishes generally operate at a routine rate that lies between the standard and maximum, and is ecologically minimal at around twice the standard level to account for about 1 L sec^{-1} swimming (foraging), specific dynamic action (assimilation) and other functions, excluding growth, spawning, extended migrations, etc. (Fry 1971, Kerr 1971, Mann 1969, Winberg 1956, Wohlschlag and Wakeman 1978). Stresses also can depress routine metabolic rates (Beamish 1964, Wohlschlag and Cameron 1967, Kloth and Wohlschlag 1972, Cech and

Wohlschlag 1975), although a depressed routine rate appears to be less definitive than scope for maximum sustained activity for species that may have a maximum swimming metabolic activity level 4-8 times standard levels (Randall 1970).

For this study the red snapper (Lutjanus campechanus) was chosen as a well known commercial and recreational species from both offshore and inshore waters. It is a relatively easy species to maintain in the laboratory.

The specific aims were to use the red snapper as a test organism:

1. To identify metabolic effects at a very low sublethal toxicant level;

2. To utilize the metabolic results at active and standard levels for detection of scope depression even though the chemical composition of the toxicants could be considered unknown;

3. For suggesting a possible biological monitoring system that could operate with or without a detailed chemical knowledge of a toxicant or mixed toxicants; and

4. For retention of energetics data on a given species of general importance in fishery and ecological considerations.

Methods

Throughout the red snapper, Lutjanus campechanus was the fish of choice. Hook-and-line fishing at offshore "snapper banks" in 80-90 meters of water about 60 km offshore at Port Aransas provided specimens for 20C experiments, while fish taken from shallower waters near the local Aransas Pass jetties and nearshore artificial reefs provided specimens for the 28C experiments. At both locations throughout the year natural salinities were very near 35 ppt.

Fish were held in live boxes with flowing seawater on board research vessels and on shore in covered outdoor or indoor tanks with flowing seawater. Frequency of feeding was sufficient to promote growth. Before experiments fish were held in temperature controlled, filtered water tanks at 35 ppt and 20C or 28C for at least 48 hrs. Fish were fasted for at least 24 hrs before respiration measurements.

The pollutant used was a biosolid from a Shell corporation waste that consisted of both solid and liquid fractions with generally known components (Anderson 1974, Siegel and Rader 1974). Since the TLM_{96} was known for the liquid phase to be 20.4% (volume), a dilution to 1% (or a volume concentration of 0.2%), was utilized throughout for comparison with controls. For experiments at 20 C, the sludge and liquid components were separated on a 0.2% v/v basis; at 28C only the liquid phase was used. The whole sludge was suspended in a loosely woven gauze bag, while the liquid was uniformly dispersed.

Preliminary experiments indicated that 0.1% v/v dilutions would also produce a measurable reduction in metabolic scope measurements. In each of the experiments in polluted waters, the fish were held 48 hrs under well oxygenated conditions before metabolic measurements ensued.

Oxygen consumption rates were measured by withdrawal of small samples for use in a Radiometer model E-5046 with a PHM 71 electrode equipped with acid-base analyzer. Following completion of a set of experimental oxygen consumption measurements, the fish were removed and lengths and weights recorded.

Resting rates were determined by using 4 13.8 cm ID (15.2 cm OD) diameter by 61 cm long acrylic tube flow-through chambers immersed in a 450 l insulated, temperature controlled aquarium equipped with a filtration system. Opaque plastic shields between the chambers and black curtains around the entire mechanism prevented visually induced excitement. Measurements of O_2 at intakes and at outlets with flow rates were made over the course of 1 or 2 days to determine minimal resting metabolism rates in well oxygenated waters.

Active metabolism rates were made in a 207 l Blazka chamber (Blazka, et al. 1960, Fry 1971) as utilized by Wohlschlag and Wakeman (1978). The entire chamber was immersed in a 3,678 l temperature-salinity controlled system, which was a contiguous part of the circular holding tank, filtration and cooling systems. Fish were maintained for one day swimming at low velocities (about 1 L sec^{-1}) prior to active measurements. After swimming in the chamber at an intermediate speed for 1 hr, the velocity was

increased gradually until the fish "broke" pace. At this instant the velocity was lowered (usually quite slightly) to the highest possible velocity at which normal swimming persisted without breaking. With this "training" regimen, the maximum sustained swimming velocity could be reproducible for each fish. The U_{max} ($\sqrt{\text{Total Lengths}}$ sec^{-1}) swimming velocity was determined at least twice to ascertain consistency, after which the fish was tested for at least 1 hr for a consistent U_{max}. Following the 1 hr or longer runs, the fish were left in the chamber at intermediate or zero velocities with oxygen rate measurements to detect any irregularities that could have resulted had the U_{max} been associated with any undesirable anaerobic metabolism.

Along with lengths, weights, oxygen consumption rates, and swimming rates in (total lengths)$^{-\frac{1}{2}}$ sec^{-1}, salinities and temperatures were recorded to 0.1 C and 0.1 ppm for a simple multiple regression at each control or experimental condition in the form:

$$\hat{Y} = a + b_w X_w + b_t X_t + b_s X_s + b_v X_v$$

where:

$\hat{Y}$ = expected O_2 consumption rate in log mgO_2hr^{-1},

a = constant,

X_w = weight in grams,

X_t = ^oC,

X_s = salinity in ppt, and

X_v = $\sqrt{L}$ sec^{-1}.

The various $\underline{b}$ values are the respective partial regression coefficients.

The respective multiple regressions are used in this study simply to indicate how salinities and temperatures can be reduced to 35 ppt and 20 C or 28 C, respectively, although the temperature and salinity controls remained near these values. The principal use of the regressions as simplified in Tables 1 and 2 is to reduce data to given "averages" of weights and swimming velocities. Similar procedures have been used by Wohlschlag and Juliano (1959), Wohlschlag and Cameron (1967), Wohlschlag and Cech (1970), and others. Regression calculation techniques are in most statistical manuals, e.g., Snedecor and Cochran (1967) or in various pretested library computer routines.

Results

Original data for the resting fish in the flow-through chambers and for the swimming fish in the Blazka chambers are in Appendix Tables 1 and 2. The multiple regression equations and associated statistics are in Appendix Tables 3 and 4 respectively. By use of these equations, the "adjusted" data for temperature, salinity, weight at 250g and averages of maximum swimming velocities are in Appendix Tables 5 and 6.

The Table 1 equations are based on the adjusted averages for temperatures of 20°C or 28°C and salinity of 35 ppt for the flow-through chamber resting metabolism and another additional "average" weight of 250 g for the swimming fish in the Blazka chamber. The actual averages and ranges of the weights, temperatures and salinities are in Table 2 for the flow-through chambers, and in Table 3 for these variables and swimming velocities in the Blazka chamber.

In order to ascertain a minimum resting rate that would be a realistic estimate of the standard rate by the Brett (1964) technique, the Appendix Figs. 1 - 5 plots (based on Table 1 Equations 1 - 5) were utilized. Similarly, for the maximum $\sqrt{L}$ sec^{-1} measurements that would be realistic, the Appendix Figs. 6 - 10 plots (based on Table 1 Equations 6 - 10) were utilized.

In Table 4 are equations 1a - 5a for the lowest estimates of the standard rates based on flow-through chamber data under various conditions; the table also includes equations 6a - 10a for highest estimates of active fishes in the Blazka chamber under comparable conditions.

Table 1. Regression equations for L. campechanus respiration, derived from multiple regression equations and adjusted to appropriate temperatures and a salinity of 35 ppt.

			Equation Number
	Flow Through Chamber Equations		
Control Water:	20C, N = 21	$Y = -0.0347 + 0.5602X_w$	(1)
	28C, N = 12	$Y = -1.2381 + 1.1973X_w$	(2)
Treated Water:	20C, N = 08*	$Y = -0.7828 + 0.8965X_w$	(3)
	20C, N = 08**	$Y = 0.2398 + 0.6530X_w$	(4)
	28C, N = 08**	$Y = -0.5214 + 0.9453X_w$	(5)
	Blazka Chamber Equations at 250g		
Control Water:	20C, N = 37	$Y = 1.2093 + 0.0455X_v$	(6)
	28C, N = 29	$Y = 1.8993 + 0.0111X_v$	(7)
Treated Water:	20C, N = 10*	$Y = 1.1708 + 0.0508X_v$	(8)
	20C, N = 27**	$Y = 1.5203 + 0.0285X_v$	(9)
	28C, N = 23**	$Y = 1.9243 + 0.0094X_v$	(10)

* = Sludge Phase

** = Liquid Phase

Table 2. Average values and ranges of variables used in regression equations for resting metabolism in flow-through chambers.

Condition		Weight (grams)		Temperature (°C)		Salinity (o/oo)	
		Average	Range	Average	Range	Average	Range
Control	21	232.2	125.0 - 676.0	20.0	19.9 - 20.0	35.4	35.0 - 35.9
	12	208.0	132.0 - 370.0	28.0	28.0	35.2	34.9 - 35.7
Treated (Sludge)	08	233.3	151.0 - 290.0	20.0	20.0	35.4	35.1 - 35.6
(Liquid)	08	211.0	130.0 - 674.0	19.9	19.8 - 20.0	35.1	34.7 - 35.4
(Liquid)	08	209.6	125.0 - 349.0	28.0	28.0 - 28.1	35.3	35.1 - 35.4

Table 3. Average values and ranges of variables used in regression equations for active metabolism in the Blazka respirometer.

Condition	N	Weight (Grams)		Temperature (^{o}C)		Salinity (o/oo)		Velocity		
		Average	Range	Average	Range	Average	Range	Average ($\sqrt{L}$ sec^{-1})	Range ($\sqrt{L}$ sec^{-1})	Range (L sec^{-1})
Control	37	211.8	128.0-676.0	20.0	19.7-20.5	35.1	34.8-35.5	14.22	03.48-20.40	0.7-4.5
	29	254.2	128.0-690.0	28.0	27.1-28.5	35.0	34.5-35.5	22.37	00.00-28.88	0.0-5.8
Treated (Sludge)	10	239.2	151.9-290.0	20.0	20.0-20.1	35.1	35.0-35.5	17.44	15.94-18.92	3.2-4.2
(Liquid)	27	368.3	129.0-787.0	20.0	19.8-20.2	35.0	34.4-36.0	12.50	00.00-19.33	0.0-4.3
(Liquid)	23	229.6	123.0-661.0	27.8	27.0-28.4	35.1	35.0-36.0	20.69	00.00-29.44	0.0-5.9

Table 4. Regression equations for L. campechanus respiration using only the lowest metabolic rates for flow-through chamber results and only the highest metabolic rates for Blazka chamber results for a 250 g. fish adjusted to the appropriate temperature and a salinity of 35 ppt. (See Appendix Figs. 1 to 10.)

		Flow-Through Chamber Equations	Equation Number
Control Water:	20C	: $Y = -0.095 + 0.5602X_w$	(1a)
	28C	: $Y = -1.290 + 1.1973X_w$	(2a)
Treated Water:	20C*	: $Y = -0.830 + 0.8965X_w$	(3a)
	20C**	: $Y = 0.200 + 0.6530X_w$	(4a)
	28C**	: $Y = -0.610 + 0.9453X_w$	(5a)
		Blazka Chamber Results	
Control Water:	20C	: $Y = 1.340 + 0.0455X_v$	(6a)
	28C	: $Y = 1.990 + 0.0111X_v$	(7a)
Treated Water:	20C*	: $Y = 1.205 + 0.0508X_v$	(8a)
	20C**	: $Y = 1.600 + 0.0285X_v$	(9a)
	28C**	: $Y = 1.970 + 0.0094X_v$	(10a)

* = Sludge Phase

** = Liquid Phase

Tables 5 and 6 include pertinent regression statistics for the Table 4 equations respectively for the standard and maximum activity estimates.

For all data, at all conditions, the U_{max} average was 21.56 $\sqrt{L}$ sec^{-1}, so that for comparative purposes, calculated maximum rates can be compared to the resting rates as in Table 7 (see also Figs. 1 and 2 below). Note that scope at 20°C is much higher than at 28°C, a summer temperature that is realistic for inshore red snappers in the smaller size ranges. However, 20°C is a more reasonable — possibly more nearly optimal — temperature for the great majority of the red snappers in the deeper waters.

From Table 7 it is apparent that the liquid waste under otherwise comparable conditions has a much greater effect in reducing the scope at 20°C than at 28°C.

Table 5. Regression statistics for L. campechanus respiration tests in the flow-through system. Final reduced equations (Table 4) adjusted to appropriate temperature and a salinity of 35 ppt.

Equation	N	Multiple Correlation Coefficient R	Std. Dev. of Estimate s_y	Standard Deviation of Regression Coefficient (s_b) and Probability (P) s_{b_w}	P
Control (20C)	21	0.94	0.0352	0.0451	0.005
Control (28C)	12	0.97	0.0395	0.0902	0.005
Treated (20C)*	08	0.94	0.0368	0.1385	0.005
Treated (20C)**	08	0.99	0.0166	0.0276	0.005
Treated (28C)**	08	0.93	0.0547	0.1581	0.005

* = Sludge Phase

** = Liquid Phase

Table 6. Regression statistics for L. campechanus respiration tests in the Blazka chamber. Final reduced equations (Table 4) adjusted to the appropriate temperature, 35 ppt. and an overall average weight of 250 g.

Equation	N	Multiple Correlation Coefficient R	Std. Dev. of Estimate s_Y	Standard Deviation of Regression Coefficient (s_b) and Probability (P) s_{b_Y}	P
Control (20C)	37	0.95	0.0792	0.0025	0.005
Control (28C)	29	0.85	0.0505	0.0013	0.005
Treated (20C)*	10	0.92	0.0256	0.0074	0.005
Treated (20C)**	27	0.96	0.0547	0.0016	0.005
Treated (28C)**	23	0.96	0.0267	0.0006	0.005

* = Sludge Phase

** = Liquid Phase

Table 7. Calculated metabolic rates and "scope" for average size (250 g) fish at appropriate temperature and a salinity of 35 ppt. The overall average U_{max} of 21.56 $\sqrt{L}$ sec^{-1} was used to calculate the maximum activity metabolic rate.

Treatment	Temperature (^oC)	Resting Rate (mgO_2 kg^{-1} hr^{-1})	Maximum Rate (mgO_2 kg^{-1} hr^{-1})	"Scope" (mgO_2 kg^{-1} hr^{-1})
Clean	20	71	838	767
Treated (Sludge)	20	84	799	715
Treated (Liquid)	20	233	655	422
Clean	28	152	678	526
Treated (Liquid)	28	182	595	413

Discussion and Conclusions

The metabolic depression of the scope for maximum sustained swimming activity at 20C or 28C in the presence of the waste materials is quite clear as summarized in Table 7. As expected, the stress costs at both temperatures involve increases in the standard and decreases in the maximum metabolic levels.

The scope depression is considerably greater at 20C (-45%) than at 28C (-21%), simply because 20C is expected to be nearer an optimal temperature for offshore waters. The red snappers from shallower water, and higher temperatures are also harder to maintain in the outdoor ponds or laboratory without a greater prevalence of morbidity, fin rot, and other gross manifestations of stress. Figs. 1 and 2 show clearly the differences between 20 and 28C.

Since the 0.2% v/v concentration has a fairly pronounced scope reduction effect after two days exposure, it is likely at 20C at least that the scope reduction would be sensitive at 0.1% v/v concentration. An earlier preliminary experiment with a different batch of biosludge was even more sensitive, although this batch of biosludge may have been less "digested" than later batches.

The system of acclimatizing the experimental fish for two days in a suspected pollutant could be made much more sensitive if the fish were held much longer to allow for slower accumulating suspected toxic substances. However, the fish would have to be fed with great care during a longer acclimatization period to be certain the food itself either did or did not

Fig. 1. Metabolic rates for a 250 gram L. campechanus tested in control and treated water at 20C. Solid line represents the metabolic rate at U_{max} (average U_{max} of 21.56) and the dashed line represents standard metabolic rates at each condition. The lightly stippled area indicates the "scope for maximum activity" for each treatment.

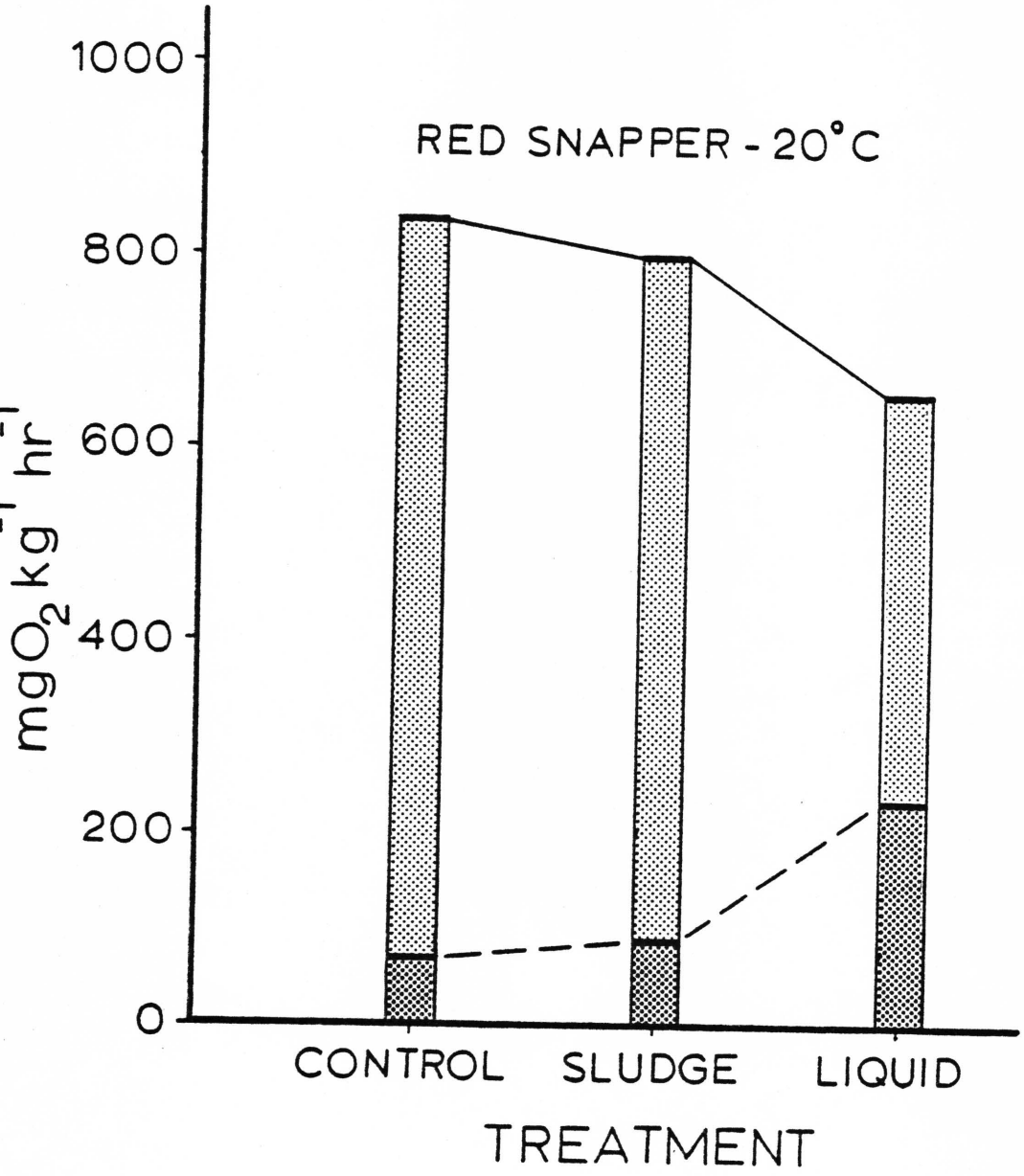
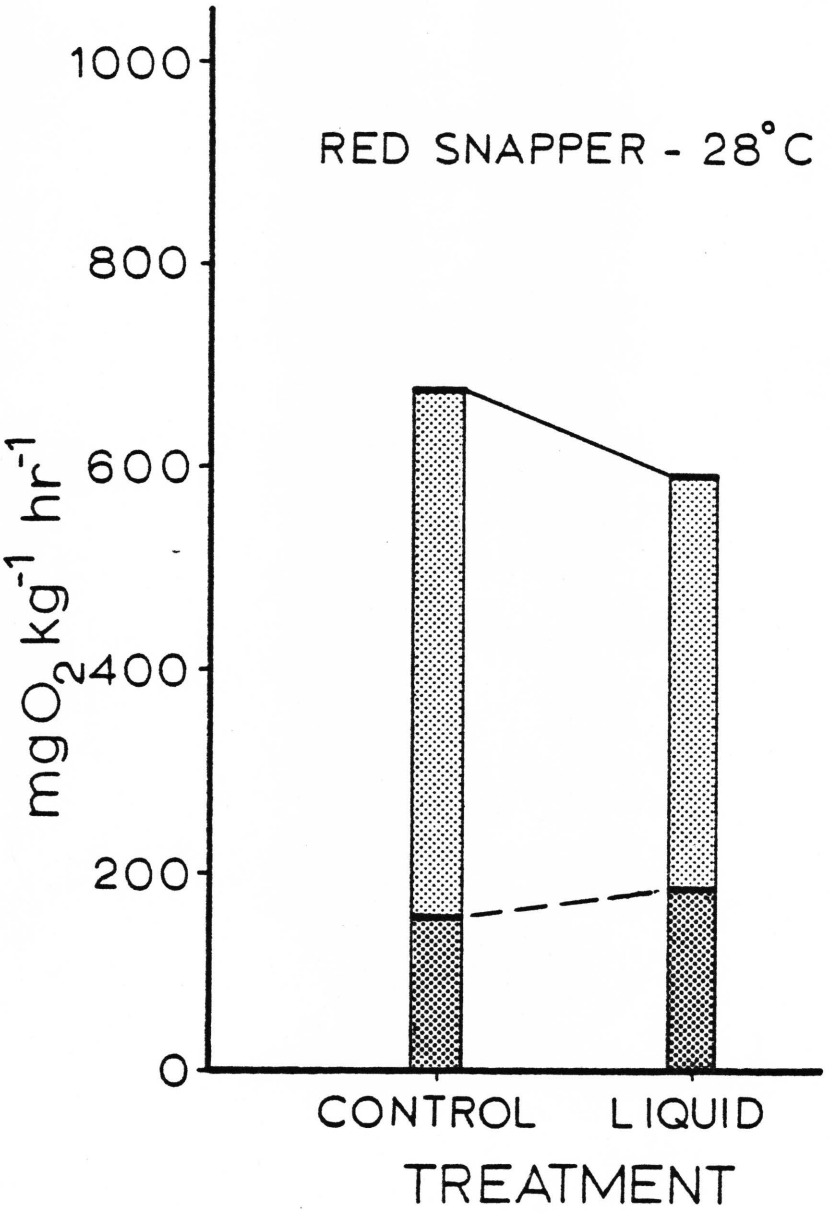

RED SNAPPER - 20°C
1000
800
600
400
200
0
$mgO_2 kg^{-1} hr^{-1}$
CONTROL
SLUDGE
LIQUID
TREATMENT

Fig. 2. Metabolic rates for a 250 gram L. campechanus tested in control and treated water at 28C. Solid line represents the metabolic rate at U_{max} (an average U_{max} of 21.56) and the dashed line represents standard metabolic rates at each condition. The lightly stippled area indicates the "scope for maximum activity" for each treatment.


RED SNAPPER - 28°C
1000
800
600
400
200
0
$mgO_2 kg^{-1} hr^{-1}$
CONTROL
LIQUID
TREATMENT

transfer the cumulative toxins.

As noted in Wohlschlag and Wakeman (1978) the maximum swimming rates also parallel the metabolic rates at maximum sustained swimming rates. This observation suggests that healthy, swimming fish, could be used to assess water quality directly without metabolic measurements. However, the swimming rates must be adjusted for the length of the fish (Webb 1975), as for the U_{max} values in this study, to avoid high swimming rates for smaller fish.

Thus, with a few simple modifications and precautions, a system of monitoring effluent effects on fish is suggested. All that would be required would be a Blazka system so that an appropriate mixture of effluent and dilution water could be adjusted to the lowest or highest desired swimming performance. For comparisons with clean waters, several seasonal sets of standard and active rate (scope) data would be required over seasonally appropriate temperatures, salinities or other conditions. Considering the fact that toxicity of an effluent can be evaluated before the biochemistry of the effluent is known, the system of using the scope as a measure of stress has considerable additional merit.

The utilization of respiratory measurements can also have great promise in fishery and general ecosystem studies based on energy measurements. Energy appears to be a common denominator for evaluating environmental optima and stresses in the sense of Cody (1974), in determining population growth—foraging relationships in the sense of Kerr (1971), or in determining

metabolic or respiration effects that are highly sensitive for heterotrophs in ecosystem models in the sense of O'Neill (1976). Energetics of systems with various diversity-stability relationships among major, highly iteroparous organisms also need to be studied in order to explain species dominance and community stability (Simenstad, et al. 1978). The various open ocean fishes such as the red snapper, are relatively long-lived and the status of their biomass and age structure would be changed appreciably by slight, chronic changes in mortality. Such changes are now suspected of having capabilities to induce dramatic effects on the stability of man and other ecosystem components (Simenstad, et al. 1978).

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APPENDIX

Tables and Figures

Appendix Table 1. Raw data used in the calculation of regression equations for fish tested in flow-through chambers (Appendix 3).

Fish No. = fish identification number

log. wt. = $\log_{10}$ weight in grams

Temp. (^{o}C) = temperature in degrees centigrade

Sal. (o/oo) = salinity expressed in parts per thousand

log. mgO_2hr^{-1} = respiration, $\log_{10}$ mgO_2hr^{-1}

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	log. mgO_2hr^{-1}
Control (20C)				
001	2.40654	20.0	35.0	1.36059
002	2.29885	20.0	35.0	1.30856
004	2.35984	20.0	35.0	1.32449
005	2.11727	20.0	35.0	1.10072
006	2.36922	20.0	35.0	1.28217
007	2.54654	20.0	35.0	1.37535
008	2.18752	20.0	35.0	1.17522
010	2.82995	20.0	35.5	1.50974
011	2.28103	20.0	35.5	1.19562
012	2.39445	20.0	35.5	1.31154
013	2.11727	20.0	35.9	1.14145
014	2.37291	20.0	35.9	1.28338
015	2.09691	20.0	35.9	1.08350
016	2.32428	20.0	35.9	1.30492
018	2.17898	20.0	35.1	1.19535
039	2.48714	19.9	35.6	1.21748
040	2.36922	19.9	35.6	1.17260
041	2.48714	19.9	35.6	1.24969
042	2.57287	19.9	35.6	1.29181
043	2.42488	20.0	35.0	1.35851
045	2.45939	20.0	35.0	1.27554

Fish No.	log wt.	Temp. (°C)	Sal. (o/oo)	log. mgO_2hr^{-1}
Control (28C)				
046	2.13672	28.0	35.0	1.28400
047	2.36549	28.0	35.0	1.55255
048	2.20140	28.0	35.0	1.34811
049	2.29003	28.0	35.1	1.54295
050	2.46389	28.0	35.1	1.68851
051	2.12057	28.0	35.1	1.36568
053	2.36361	28.0	34.9	1.62603
054	2.29447	28.0	34.9	1.52943
055	2.56820	28.0	35.5	1.81578
056	2.24055	28.0	35.5	1.37199
057	2.41500	28.0	35.5	1.60788
058	2.35603	28.0	35.7	1.53567
Treated, Sludge (20C)				
027	2.17898	20.0	35.6	1.22453
028	2.38739	20.0	35.6	1.33985
023	2.42488	20.0	35.1	1.38364
024	2.39445	20.0	35.1	1.34908
025	2.25042	20.0	35.1	1.23477
026	2.45332	20.0	35.1	1.46494
019	2.39094	20.0	35.6	1.44716
021	2.46240	20.0	35.6	1.46165
Treated, Liquid (20C)				
037	2.20140	20.0	35.3	1.27623
038	2.26482	20.0	35.3	1.28533

Fish No.	log wt.	Temp. (°C)	Sal. (o/oo)	log. mgO_2hr^{-1}
Treated, Liquid (20C) Cont.				
033	2.26717	20.0	35.4	1.13925
034	2.11394	20.0	35.4	1.08422
035	2.39445	20.0	35.4	1.25042
036	2.14922	20.0	35.4	1.09026
030	2.82867	19.8	34.7	1.55206
031	2.38202	19.8	34.7	1.26055
Treated, Liquid (28C)				
059	2.23045	28.0	35.3	1.40019
060	2.35411	28.0	35.3	1.46613
061	2.36549	28.0	35.3	1.61542
062	2.25042	28.0	35.1	1.49248
063	2.36173	28.0	35.1	1.70817
064	2.54283	28.1	35.4	1.79029
065	2.09691	28.1	35.4	1.41280
066	2.36922	28.1	35.4	1.68789

Appendix Table 2. Raw data used in the calculation of regression equations for fish tested in the Blazka chamber (Appendix 3).

Fish No. = fish identification number

log. wt. = $\log_{10}$ weight in grams

Temp. (^{o}C) = temperature in degress centigrade

Sal. (o/oo) = salinity in parts per thousand

V ($\sqrt{L}$ sec^{-1}) = velocity in square root of lengths per second

log. mgO_2hr^{-1} = respiration, $\log_{10}$ mgO_2hr^{-1}

*by a respiration measurement indicates that the reading was made at an actual U_{max} for that fish.

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V ($\sqrt{L}$ sec^{-1})	log. $mgO_2 hr^{-1}$
Control (20C), U_{max} avg. = 17.70					
119	2.10721	20.0	34.9	15.20*	1.71450
120	2.12057	19.7	35.0	16.50*	1.79064
121	2.12057	19.7	35.0	17.80	1.84942
122	2.12057	19.7	35.0	14.20	1.64365
123	2.14613	20.0	35.0	14.50	1.60767
124	2.14613	20.0	35.0	20.40*	1.83651
125	2.18752	20.0	35.0	15.60	1.72558
126	2.18752	20.0	35.0	17.90*	1.79900
127	2.18752	20.0	35.0	17.00*	1.79518
128	2.19312	20.0	35.0	13.90	1.79211
129	2.19312	20.0	35.0	19.50	1.89642
131	2.27416	20.5	35.0	19.10*	1.97230
132	2.31806	19.9	35.0	18.58*	1.88835
133	2.31806	19.9	35.0	14.29	1.75557
134	2.32015	20.0	35.0	14.54	1.67825
135	2.31806	20.1	35.5	05.24	1.56820
136	2.31806	20.1	35.5	04.29	1.54220
137	2.32015	20.0	35.0	19.88	1.97179
138	2.35984	20.0	34.8	16.97	1.97722
139	2.82995	20.2	35.5	15.15	2.37157
140	2.55388	20.0	35.0	15.87	1.98981
141	2.55388	20.0	35.0	19.05*	2.14919
142	2.42651	20.0	35.0	17.60*	1.97722

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V ($\sqrt{L}$ sec^{-1})	log. mgO_2hr^{-1}
Control (20C), U_{max} avg. = 17.70 (Cont.)					
143	2.40140	19.9	35.0	14.73	2.01941
144	2.40140	19.9	35.0	15.71*	2.05507
146	2.38917	20.0	35.0	13.63	1.76253
147	2.38917	20.0	35.0	17.67*	2.04653
148	2.38917	20.0	35.0	15.22	1.97987
149	2.38917	20.0	35.0	18.16*	2.07780
150	2.37291	20.0	35.0	14.15	1.87967
151	2.37291	20.0	35.0	18.05*	2.00359
152	2.55388	20.0	35.0	03.70	1.46687
153	2.42651	20.2	35.5	04.02	1.44420
154	2.37291	20.0	35.3	03.90	1.32675
155	2.36922	20.0	35.0	03.48	1.34064
157	2.35984	20.1	35.0	03.99	1.26387
160	2.25285	20.4	35.5	16.85*	1.87927
Control (28C), U_{max} avg. = 26.06					
059	2.83885	27.3	35.0	00.00	2.25310
060	2.83885	28.0	34.9	16.97	2.49799
061	2.83885	28.0	34.9	23.76*	2.47780
063	2.34635	27.8	35.0	19.03	2.18041
064	2.34635	27.8	35.0	23.42*	2.20243
065	2.34635	27.1	35.0	23.42*	2.17569
066	2.57403	27.9	34.5	17.37	2.23355
067	2.57403	28.0	34.5	27.37*	2.38819
068	2.57403	28.2	34.5	27.37*	2.34143
069	2.41497	28.0	34.5	23.18	2.17863

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V ($\sqrt{L}$ sec^{-1})	log. mgO_2hr^{-1}
Control (28C), U_{max} avg. = 27.06 (Cont.)					
070	2.35793	27.9	35.0	17.82	2.01611
071	2.35793	28.0	35.0	27.72*	2.15247
072	2.35793	28.1	35.0	20.29	2.17455
073	2.35793	28.1	35.0	28.21*	2.18233
074	2.64738	28.2	35.0	00.00	2.06066
075	2.10721	28.0	35.0	25.08*	2.06288
076	2.39967	28.0	35.0	26.00	2.17534
077	2.39967	28.0	35.0	27.00*	2.24667
078	2.27416	27.5	35.0	18.02	2.07203
079	2.27416	27.5	35.0	27.99*	2.09764
081	2.30535	28.4	35.5	25.63*	2.04999
082	2.15229	28.2	35.5	26.58*	1.99021
083	2.46538	28.1	35.0	21.34	2.13111
084	2.46538	28.1	35.0	25.51*	2.21519
085	2.31175	27.5	35.0	23.03	2.16699
088	2.20140	28.2	35.5	23.72	1.87967
089	2.13672	28.4	35.0	25.86	1.89470
090	2.13672	28.4	35.0	28.21*	1.95554
091	2.35025	28.5	35.0	28.88*	2.10483
Treated Sludge (20C), U_{max} avg. = 17.28					
161	2.18169	20.0	35.5	18.92*	1.81023
162	2.26007	20.0	35.0	16.09*	1.87558
163	2.37291	20.0	35.0	18.78*	2.11069
164	2.38202	20.0	35.0	16.90*	2.00557
166	2.38561	20.0	35.0	17.04*	1.98682

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V ($\sqrt{L}$ sec^{-1})	log. mgO_2hr^{-1}
Treated Sludge (20C), U_{max} avg. = 17.28 (Cont.)					
167	2.40312	20.1	35.0	15.94*	1.98191
168	2.43775	20.0	35.5	17.50*	1.97433
170	2.44716	20.0	35.0	18.87	2.20238
171	2.45484	20.0	35.0	17.89*	2.09423
172	2.46240	20.0	35.0	16.43*	2.09947
Treated Liquid (20C), U_{max} avg. = 17.05					
185	2.48144	20.0	35.0	00.00	1.50325
187	2.51188	20.0	35.0	10.14	1.80828
189	2.51188	20.0	35.0	14.41	1.95525
190	2.51055	20.0	35.0	00.00	1.63124
191	2.74974	20.0	35.0	14.06*	2.26060
192	2.74974	20.0	35.0	00.00	1.82556
194	2.89265	20.0	35.0	10.63	2.22300
196	2.89265	20.0	35.0	18.90*	2.40157
197	2.57287	20.1	35.0	00.00	1.59627
199	2.57287	20.1	35.0	13.68	1.95751
200	2.57287	20.0	35.0	17.89	2.11032
201	2.89597	20.0	35.0	00.00	1.99782
202	2.89597	20.0	35.0	10.78	2.19524
203	2.89597	20.0	35.0	13.18	2.31481
204	2.89597	20.0	35.0	14.98	2.34573
206	2.76268	20.0	35.0	11.88	2.04739
207	2.76268	20.0	35.0	14.71	2.23967
208	2.76268	20.0	35.0	19.23	2.41576

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V ($\sqrt{L}$ sec^{-1})	log. mgO$_2$hr^{-1}
Treated Liquid (20C), U_{max} avg. = 17.05					
173	2.11059	20.1	34.9	11.36	1.56573
174	2.11059	20.1	34.9	16.61*	1.70200
175	2.15836	20.0	35.0	19.33*	1.89120
177	2.25285	20.2	36.0	17.12*	1.95463
179	2.27875	20.0	35.0	17.78*	1.94758
180	2.31597	19.8	35.0	18.01*	1.98046
181	2.37840	20.0	35.0	12.55	1.89878
182	2.37840	20.0	35.0	18.34*	2.04052
183	2.38739	19.8	34.5	16.20*	2.07361
Treated Liquid (28C), U_{max} avg. = 26.10					
092	2.37107	28.2	35.0	24.34	2.13599
093	2.36549	27.8	35.0	29.44*	2.15637
094	2.23805	27.8	35.0	20.16	1.96303
095	2.36736	28.0	35.0	24.78	2.11621
096	2.24551	27.0	35.0	23.61	1.96123
097	2.24551	27.0	35.0	27.39	1.98218
098	2.24551	27.0	35.0	25.97*	2.01828
100	2.38021	28.2	35.0	27.83*	2.15978
101	2.38021	28.2	35.0	25.30	2.19145
102	2.82020	28.1	35.0	00.00	2.30638
103	2.82020	27.8	35.0	18.67*	2.48636
104	2.82020	27.8	35.0	00.00	2.26867
106	2.56110	28.2	35.0	25.40*	2.29108
107	2.08991	28.4	35.0	22.20	1.85352
108	2.08991	28.4	35.0	27.40*	1.87806

Fish No.	log. wt.	Temp. (°C)	Sal. (o/oo)	V ($\sqrt{L}$ sec^{-1})	log. mgO_2hr^{-1}
109	2.23805	27.8	35.0	23.40	2.00941
110	2.23850	27.8	35.0	27.00*	2.09356
111	2.30535	27.1	35.0	00.00	1.83174
112	2.32222	27.2	35.0	28.50*	2.15369
113	2.30535	28.1	36.0	00.00	1.86900
114	2.30535	27.2	36.0	23.80	2.05622
115	2.30535	27.2	36.0	24.70*	2.05591
116	2.24304	28.0	35.0	25.90	2.05038

Appendix Table 3. Original equations for L. campechanus respiration, all variables included.

Flow-Through Chamber Equations	Equation Number
Control Water:	
20C, N = 21 : $Y = -21.29070 + 0.56023X_w + 1.09653X_t - 0.01928X_s$	(1c)
28C, N = 12 : $Y = -2.48221 + 1.19729X_w + 0.14117X_t - 0.07739X_s$	(2c)
Treated Water:	
*20C, N = 08 : $Y = -3.11759 + 0.89645X_w + 0.00000X_t + 0.06671X_s$	(3c)
**20C, N = 08 : $Y = -46.86570 + 0.65339X_w + 4.77874X_t - 1.38491X_s$	(4c)
**28C, N = 08 : $Y = -29.44410 + 0.94532X_w + 1.76070X_t - 0.58220X_s$	(5c)
Blazka Chamber Equations	
Control Water:	
20C, N = 37 : $Y = -6.81392 + 0.81048X_w - 0.12605X_t + 0.24548X_s + 0.04562X_v$	(6c)
28C, N = 29 : $Y = 5.43811 + 0.73691X_w - 0.09823X_t - 0.07301X_s + 0.01109X_v$	(7c)
Treated Water:	
*20C, N = 10 : $Y = 8.23674 + 0.91953X_w - 0.03468X_t - 0.24507X_s + 0.05080X_v$	(8c)
**20C, N = 27 : $Y = 4.46818 + 0.76345X_w - 0.30058X_t + 0.03531X_s + 0.02822X_v$	(9c)
**28C, N = 23 : $Y = -1.21438 + 0.89113X_w + 0.02223X_t + 0.01083X_s + 0.00942X_v$	(10c)

* = Sludge Phase; ** = Liquid Phase.

Appendix Table 4a. Regression statistics for L. campechanus respiration tests in flow-through system. Original equations (Appendix Table 3) with all variables included.

Equation Number	N	Multiple Correlation Coefficient	Std. Error of Estimate	Standard Errors of Regression Coefficient (s_b) and Probability (P)					
		R	s_y	s_{b_w}	P	s_{b_t}	P	s_{b_s}	P
(1c)	21	0.94	0.0373	0.0509	0.005	0.2336	0.005	0.0243	n.s.
(2c)	12	0.97	0.0442	0.1088	0.005	0.0041	n.s.	0.0517	n.s.
(3c)	08	0.93	0.0403	0.1532	0.005	0.0000	n.s.	0.0576	n.s.
(4c)	08	0.99	0.0194	0.0426	0.005	0.5657	0.005	0.1676	0.005
(5c)	08	0.94	0.0670	0.1946	0.005	0.7367	0.025	0.3059	0.05

Appendix Table 4b. Regression statistics for L. campechanus respiration tests in the Blazka system. Original equations (Appendix Table 3) with all variables included.

Equation Number	N	Multiple Correlation Coefficient	Std. Error of Estimate	Standard Errors of Regression Coefficient (s_b) and Probability (P)							
		R	s_y	s_{b_w}	P	s_{b_t}	P	s_{b_s}	P	s_{b_v}	P
(6c)	37	0.96	0.0757	0.0902	0.005	0.0974	n.s.	0.0889	0.005	0.0028	0.005
(7c)	29	0.94	0.0535	0.0692	0.005	0.0328	0.005	0.0473	n.s.	0.0018	0.005
(8c)	10	0.98	0.0323	0.1293	0.005	0.3839	n.s.	0.0591	0.005	0.0119	0.005
(9c)	27	0.97	0.0632	0.0471	0.005	0.1983	n.s.	0.0607	n.s.	0.0020	0.005
(10c)	23	0.99	0.0287	0.0354	0.005	0.0132	n.s.	0.0189	n.s.	0.0007	0.005

Appendix Table 5. Fish identification number, weight and respiration for Lutjanus campechanus tested in flow-through chambers. Adjusted rates.

Fish No. = fish identification number

Log. Wt. = $\log_{10}$ weight in grams

log mgO_2hr^{-1} = respiration, $\log_{10}$ mgO_2hr^{-1}

Fish No.	Log. Wt.	Log. mgO_2hr^{-1}	Fish No.	Log Wt.	Log. mgO_2hr^{-1}
Control (20C)					
001	2.40654	1.36059	014	2.37291	1.30065
002	2.29885	1.30856	015	2.09691	1.10085
004	2.35984	1.32449	016	2.32428	1.32227
005	2.11727	1.10072	018	2.17898	1.19723
006	2.36922	1.28217	039	2.48714	1.33870
007	2.54654	1.37585	040	2.36922	1.29382
008	2.18752	1.17522	041	2.48714	1.37091
010	2.82995	1.51938	042	2.57287	1.41303
011	2.28103	1.20526	043	2.42488	1.35851
012	2.39445	1.32118	0.45	2.45939	1.27554
013	2.11727	1.15880			
Control (28C)					
046	2.13672	1.28400	052	2.36361	1.61829
047	2.36549	1.55255	054	2.29447	1.52169
048	2.20140	1.34811	055	2.56820	1.85448
049	2.29003	1.55069	056	2.24055	1.41069
050	2.46389	1.69625	057	2.41500	1.64658
051	2.12057	1.37342	058	2.35603	1.58984
Treated, Sludge (20C)					
027	2.17898	1.18450	025	2.25042	1.22810
028	2.38739	1.29982	026	2.45332	1.45827
023	2.42488	1.37697	019	2.39094	1.40713
024	2.39445	1.34241	021	2.46240	1.42162

Fish No.	Log. Wt.	Log. mgO_2hr^{-1}	Fish No.	Log. Wt.	Log. mgO_2hr^{-1}
Treated, Liquid (20C)					
037	2.20140	1.69170	035	2.39445	1.80438
038	2.26482	1.70080	036	2.14922	1.64422
033	2.26717	1.69321	030	2.82867	2.09234
034	2.11394	1.63818	031	2.38202	1.80083
Treated, Liquid (28C)					
059	2.23045	1.57485	063	2.36173	1.76639
060	2.35411	1.64079	064	2.54283	1.84710
061	2.36549	1.79008	065	2.09691	1.46961
062	2.25042	1.55070	066	2.36922	1.74470

Appendix Table 6. Fish identification number, weight, swimming velocity, and respiration for Lutjanus campechanus tested in the Blazka chamber. Adjusted rates.

Fish No. = fish identification number

Log. Wt. = $\log_{10}$ weight in grams

V ($\sqrt{L}$ sec^{-1}) = swimming velocity expressed as the square root of total length per second.

(adj. 250 g) Log. mgO_2hr^{-1} = respiration, $\log_{10}$ mgO_2hr^{-1}, adjusted to the average weight (250 grams) of fish tested.

(adj. U_{max}) Log. mgO_2hr^{-1} = respiration, $\log_{10}mgO_2hr^{-1}$, adjusted to the average U_{max} for each experimental condition. U_{max} for each condition listed with heading for that group of experiments.

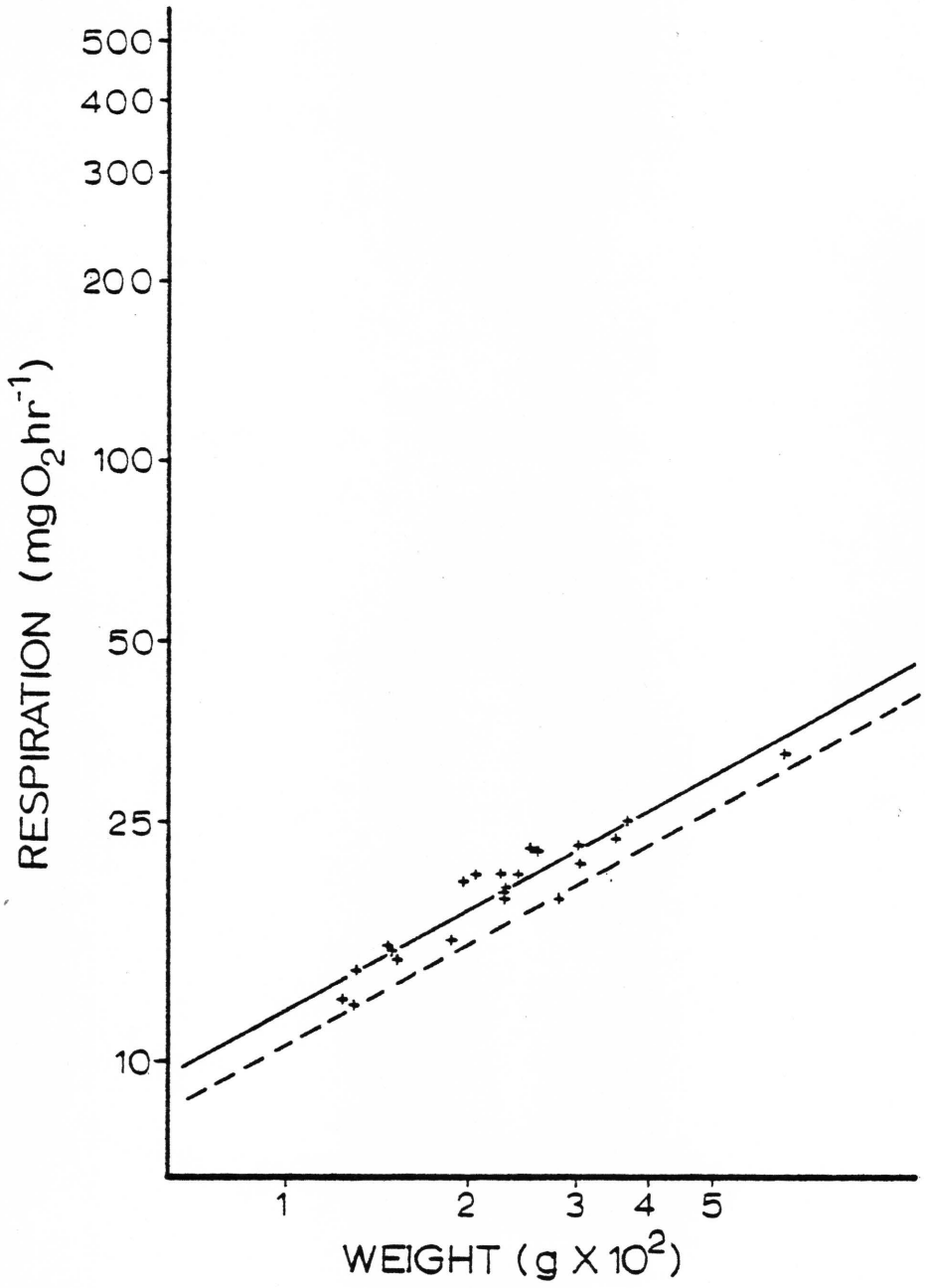
Fish No.	Log Wt.	V ($\sqrt{L}$ sec^{-1})	(adj. 250 g) Log. mgO_2hr^{-1}	(adj. U_{max}) Log. mgO_2hr^{-1}
Control (20C), U_{max} = 17.70				
119	2.10721	15.20	1.97468	1.85310
120	2.12057	16.50	1.97762	1.80756
121	2.12057	17.80	2.03640	
122	2.12057	14.20	1.83063	
123	2.14613	14.50	1.81176	
124	2.14613	20.40	2.04060	1.71334
125	2.18752	15.60	1.89612	
126	2.18752	17.90	1.96954	1.78988
127	2.18752	17.00	1.96572	1.82711
128	2.19312	13.90	1.95811	
129	2.19312	19.50	2.06242	
131	2.27416	19.10	2.13565	1.97146
132	2.31806	18.58	1.94048	1.83559
133	2.31806	14.29	1.80770	
134	2.32015	14.54	1.74130	
135	2.31806	05.24	1.52281	
136	2.31806	04.29	1.49681	
137	2.32015	19.88	2.03484	
138	2.35984	16.97	2.05720	
139	2.82995	15.15	1.92390	
140	2.55388	15.87	1.86342	
141	2.55388	19.05	2.02280	2.08760
142	2.42651	17.60	1.95406	1.98178
143	2.40140	14.73	2.00400	

Fish No.	Log. Wt.	V ($\sqrt{L}$ sec^{-1})	(adj. 250 g) Log. mgO_2hr^{-1}	(adj. U_{max}) Log. mgO_2hr^{-1}
144	2.40140	15.71	2.03966	2.13324
146	2.38917	13.63	2.02280	2.08760
147	2.38917	17.67	2.05364	2.04790
148	2.38917	15.22	1.98698	
149	2.38917	18.16	2.08491	2.05681
150	2.37291	14.15	1.89996	
151	2.37291	18.05	2.02388	1.98762
152	2.55388	03.70	1.34048	
153	2.42651	04.02	1.32351	
154	2.37291	03.90	1.27340	
155	2.36922	03.48	1.36392	
157	2.35984	03.99	1.30736	
160	2.25285	16.85	1.92454	1.84573
Control (28C), U_{max} = 26.06				
059	2.83885	00.00	1.85943	
060	2.83885	16.97	2.16578	
061	2.83885	23.76	2.14559	2.49601
063	2.34635	19.03	2.19878	
064	2.34635	23.42	2.22080	2.21206
065	2.34635	23.42	2.12530	2.11656
066	2.57403	17.37	2.05746	
067	2.57403	27.37	2.22192	2.33715
068	2.57403	27.37	2.19481	2.29039
069	2.41497	23.18	2.12957	2.17406
070	2.35793	17.82	2.03577	
071	2.35793	27.72	2.18195	2.13406

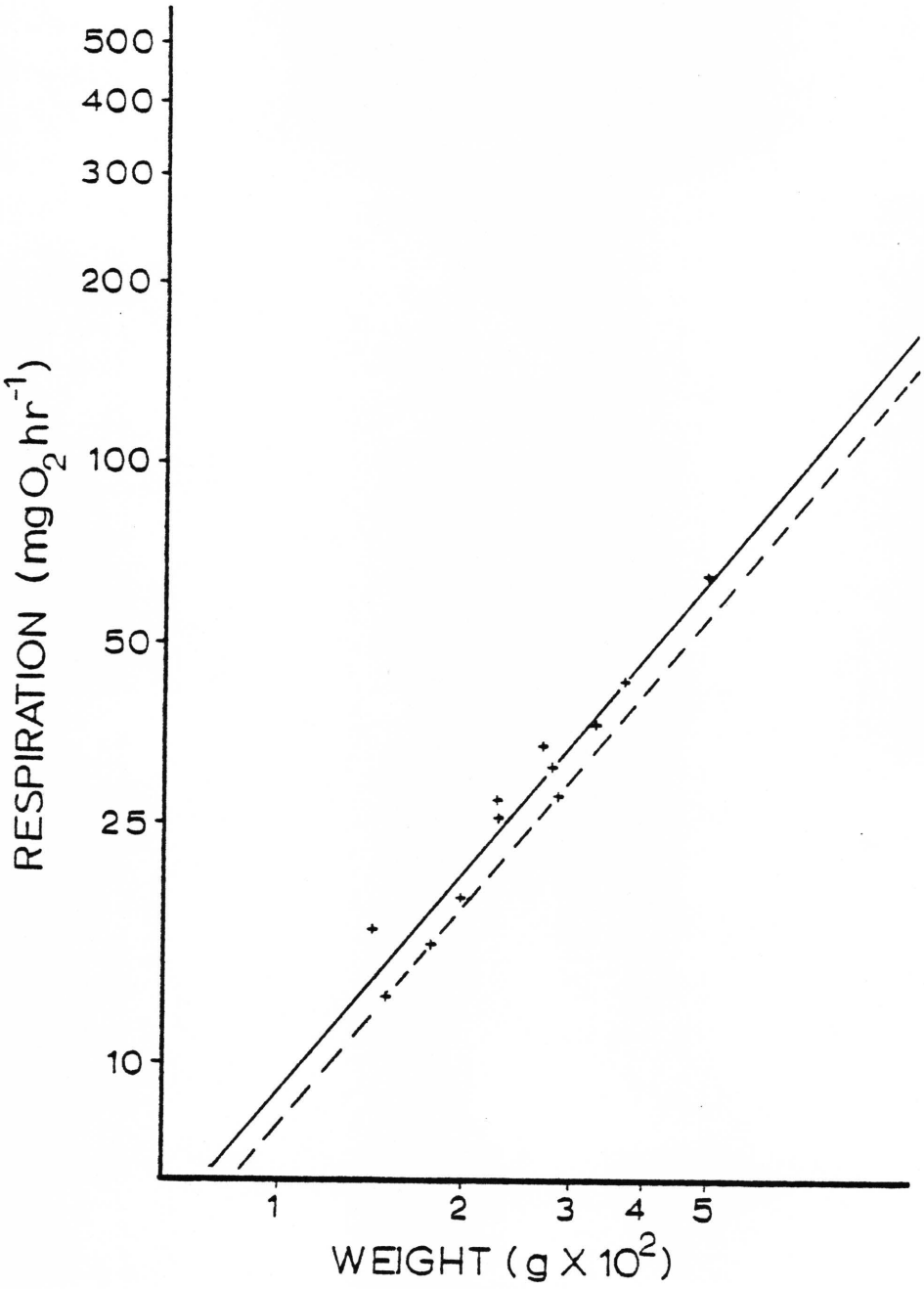
Fish No.	Log. Wt.	V ($\sqrt{L}$ sec^{-1})	(adj. 250 g) Log. mgO_2hr^{-1}	(adj. U_{max}) Log. mgO_2hr^{-1}
072	2.35793	20.29	2.21385	
073	2.35793	28.21	2.22163	2.16831
074	2.64738	00.00	1.89650	
075	2.10721	25.08	2.27712	2.07375
076	2.39967	26.00	2.17407	
077	2.39967	27.00	2.24540	2.23625
078	2.27416	18.02	2.11412	
079	2.27416	27.99	2.13973	2.02712
081	2.30535	25.63	2.19402	2.13056
082	2.15229	26.58	2.22739	2.04060
083	2.46538	21.34	2.09123	
084	2.46538	25.51	2.17531	2.23111
085	2.31175	23.03	2.18138	
088	2.20140	23.72	2.08066	1.96178
089	2.13672	25.86	2.12649	
090	2.13672	28.21	2.18733	1.97099
091	2.35025	28.88	2.18909	2.12268
Treated Sludge (20C), U_{max} = 17.28				
161	2.18169	18.92	2.13162	1.84946
162	2.26007	16.09	2.00236	1.93603
163	2.37291	18.78	2.13371	2.03449
164	2.38202	16.90	2.02021	2.02487
166	2.38561	17.04	1.99816	1.99901
167	2.40312	15.94	1.98062	2.05345
168	2.43775	17.50	2.06026	2.08569
170	2.44716	18.87	2.15712	

Fish No.	Log. Wt.	V ($\sqrt{L}$ sec^{-1})	(adj. 250 g) Log. mgO_2hr^{-1}	(adj. U_{max}) Log. mgO_2hr^{-1}
171	2.45484	17.89	2.04191	2.06324
172	2.46240	16.43	2.04020	2.14265
Treated Liquid (20C), U_{max} = 17.05				
185	2.48144	00.00	1.43950	
187	2.51183	10.14	1.72129	
189	2.51188	14.41	1.86826	
190	2.51055	00.00	1.54527	
191	2.74974	14.06	1.99202	2.34498
194	2.89265	10.63	1.84531	
196	2.89265	18.90	2.02388	2.34936
197	2.57287	00.00	1.49278	
199	2.57287	13.68	1.85402	
200	2.57287	17.89	1.97677	
201	2.89597	00.00	1.61760	
202	2.89597	10.78	1.81502	
203	2.89597	13.18	1.93459	
204	2.89597	14.98	1.96551	
206	2.76268	11.88	1.76893	
207	2.76268	14.71	1.96121	
208	2.76268	19.23	2.13730	
173	2.11059	11.36	1.81870	
174	2.11059	16.61	1.95497	1.74801
175	2.15836	19.33	2.07411	1.82686
177	2.25285	17.12	2.09021	1.97746
179	2.27875	17.78	2.03858	1.92698

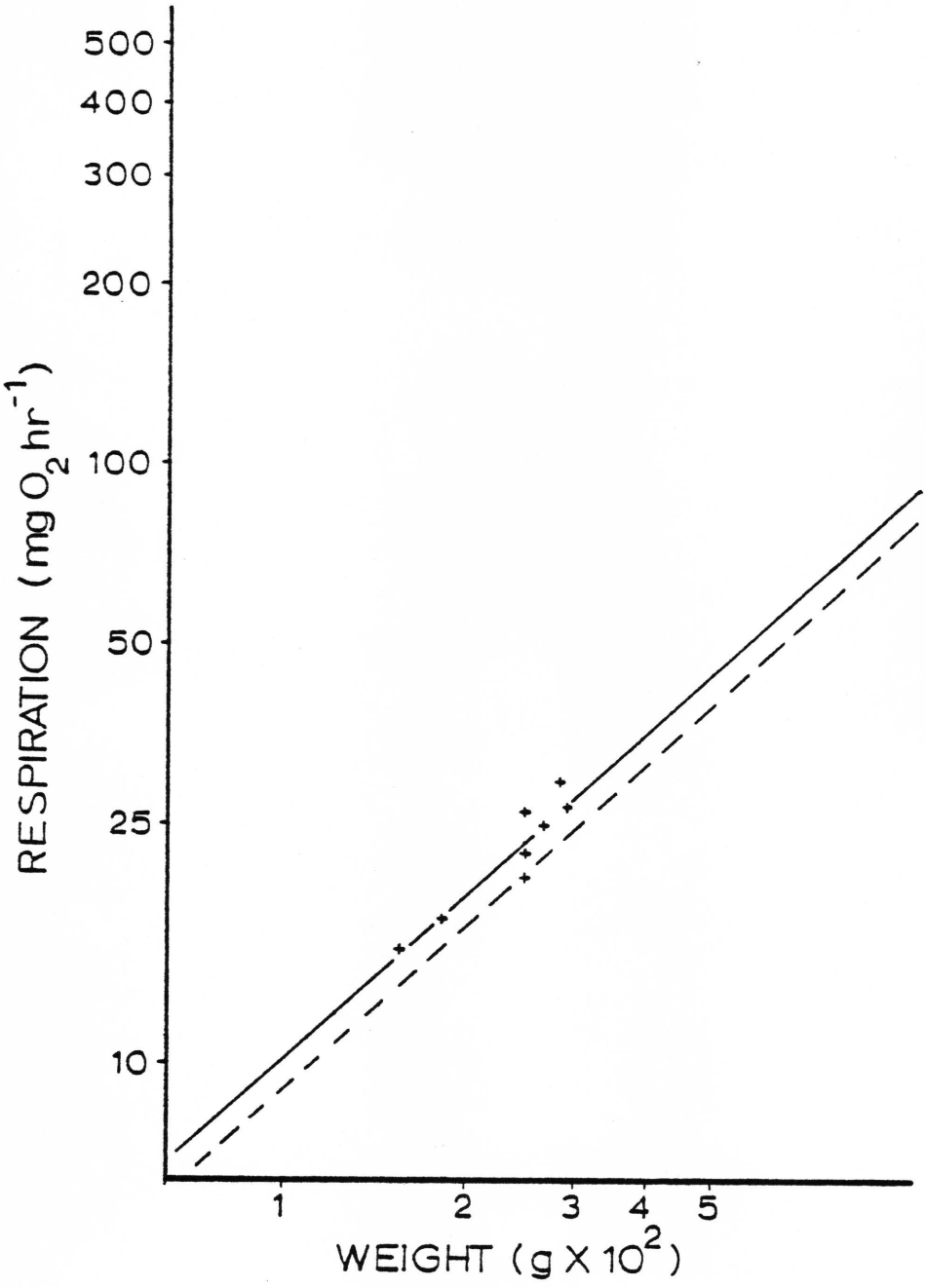
Fish No.	Log. Wt.	V ($\sqrt{L}$ sec^{-1})	(adj. 250 g) Log. mgO_2hr^{-1}	(adj. U_{max}) Log. mgO_2hr^{-1}
180	2.31597	18.01	1.98292	1.89325
181	2.37840	12.55	1.91370	
182	2.37840	18.34	2.05544	2.00412
183	2.38739	16.20	2.03920	2.05514
Treated Liquid (28C), U_{max} = 26.10				
092	2.37107	24.34	2.15548	
093	2.36549	29.44	2.18974	2.12936
094	2.23805	20.16	2.10996	
095	2.36736	24.78	2.14346	
096	2.24551	23.61	2.11929	
097	2.24551	27.39	2.13929	
098	2.24551	25.97	2.17634	2.04173
100	2.38021	27.83	2.17113	2.13903
101	2.38021	25.30	2.20280	
102	2.82020	00.00	1.92787	
103	2.82020	18.67	2.11452	2.56080
104	2.82020	00.00	1.89683	
106	2.56110	25.40	2.14123	2.29322
107	2.08991	22.20	2.11912	
108	2.08991	27.40	2.14366	1.85692
109	2.23805	23.40	2.15634	
110	2.23805	27.00	2.24009	2.08953
111	2.30535	00.00	1.93426	
112	2.32222	28.50	2.23895	2.14886
113	2.30535	00.00	1.93845	
114	2.30535	23.00	2.14568	
115	2.30535	24.70	2.14537	2.07605
116	2.24304	25.90	2.18842	



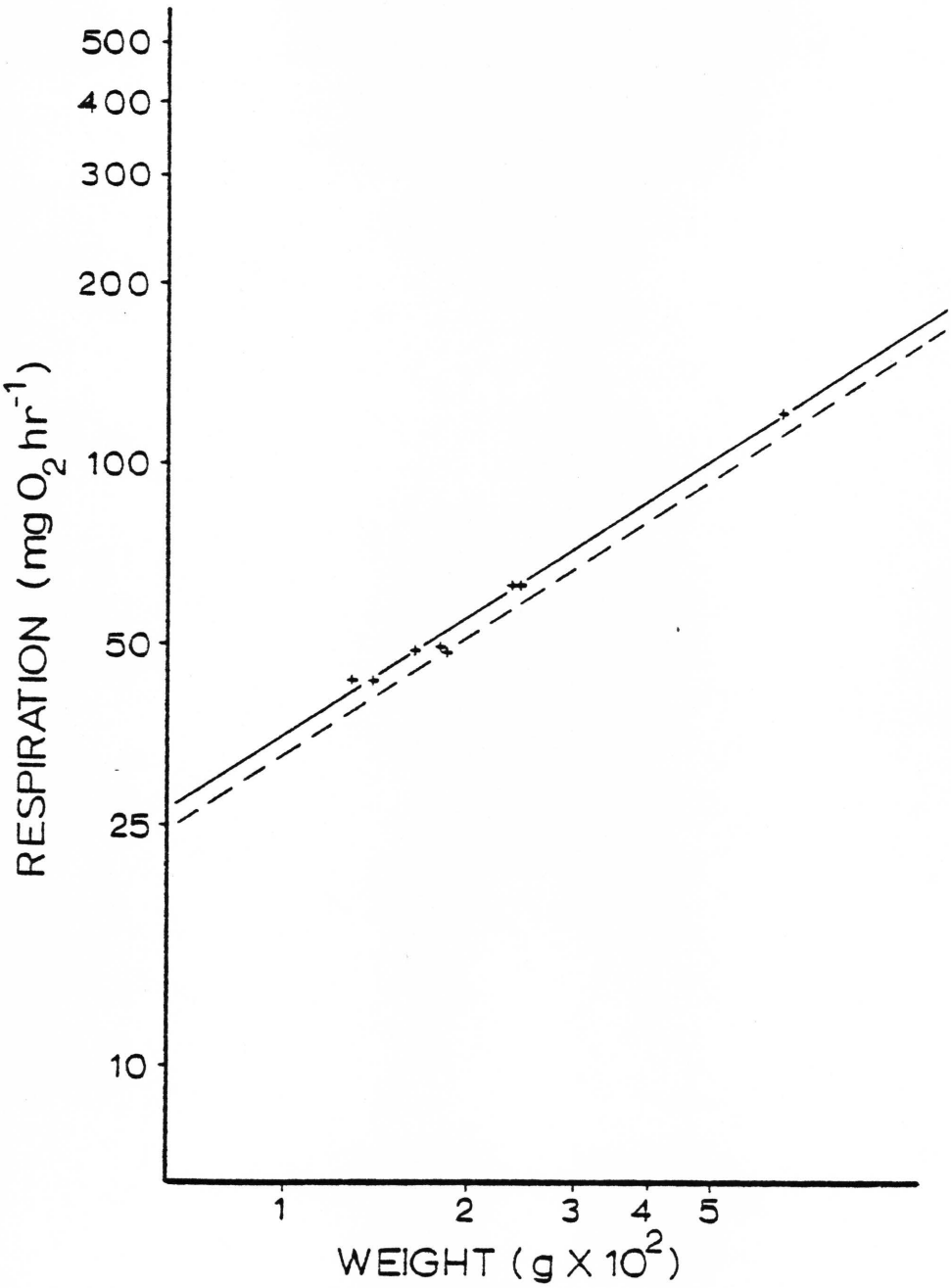
Appendix Fig. 1. Respiration and weight plot at 20C and 35 ppt. for Lutjanus campechanus in control water. Crosses represent observed data. Solid line drawn from equation 1a in Table 1. Dashed line is for estimate of the standard level drawn parallel to the resting respiration line through the lowest measured values. Resting measurements made in flow-through chambers.



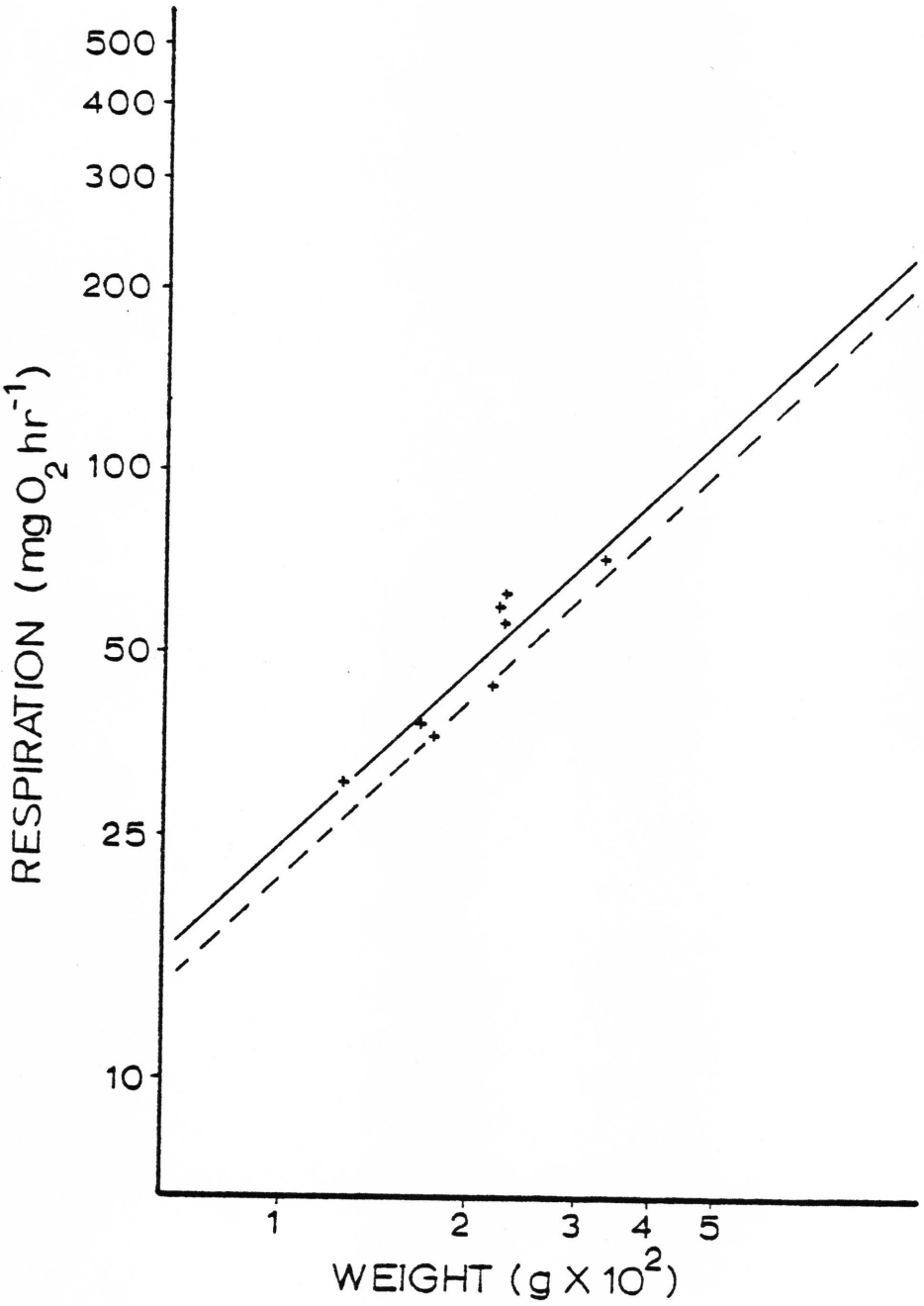
Appendix Fig. 2. Respiration and weight plot at 28C and 35 ppt. for Lutjanus campechanus in control water. Crosses represent observed data. Solid line drawn from equation 2a in Table 1. Dashed line is for estimate of the standard level drawn parallel to the resting respiration line through the lowest measured values. Resting measurements made in flow-through chambers.



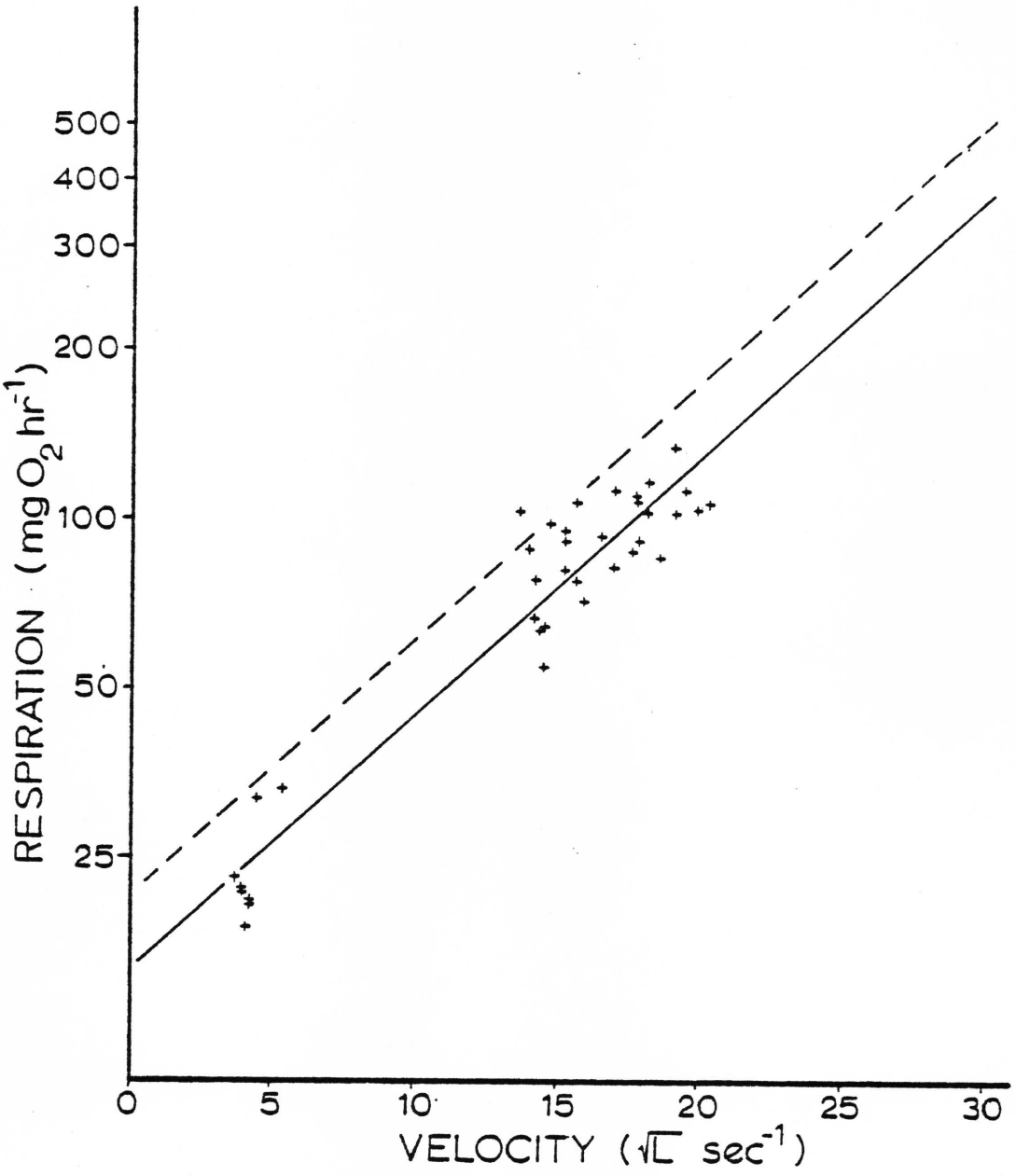
Appendix Fig. 3. Respiration and weight plot at 20C and 35 ppt. for Lutjanus campechanus in polluted (sludge phase) water. Crosses represent observed data. Solid line drawn from equation 3a in Table 1. Dashed line is for estimate of the standard level drawn parallel to the resting respiration line through the lowest measured values. Resting measurements made in flow-through chambers.



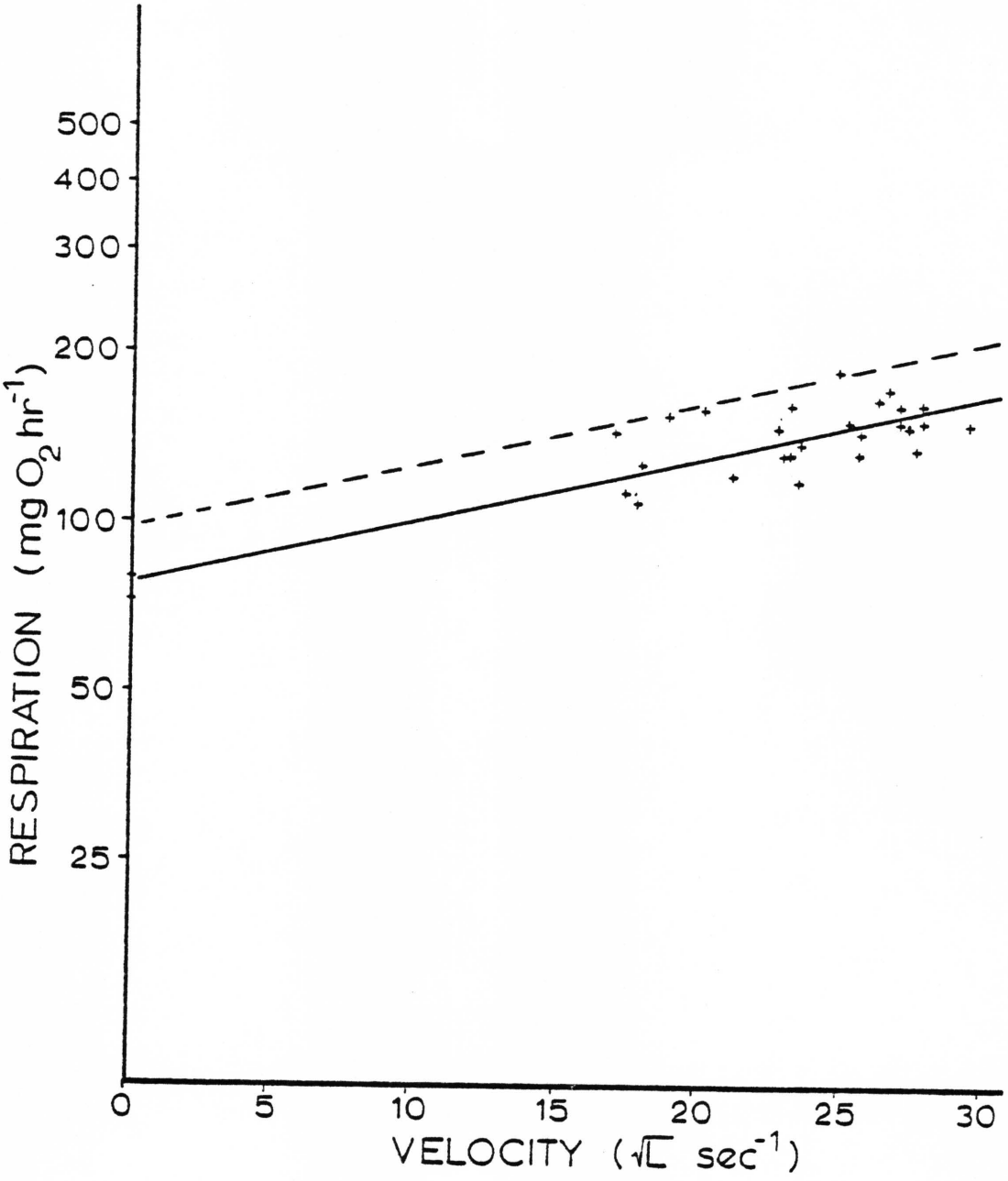
Appendix Fig. 4. Respiration and weight plot at 20C and 35 ppt. for Lutjanus campechanus in polluted (liquid phase) water. Crosses represent observed data. Solid line drawn from equation 4a in Table 1. Dashed line is for estimate of the standard level drawn parallel to the resting respiration line through the lowest measured values. Resting measurements made in flow-through chambers.



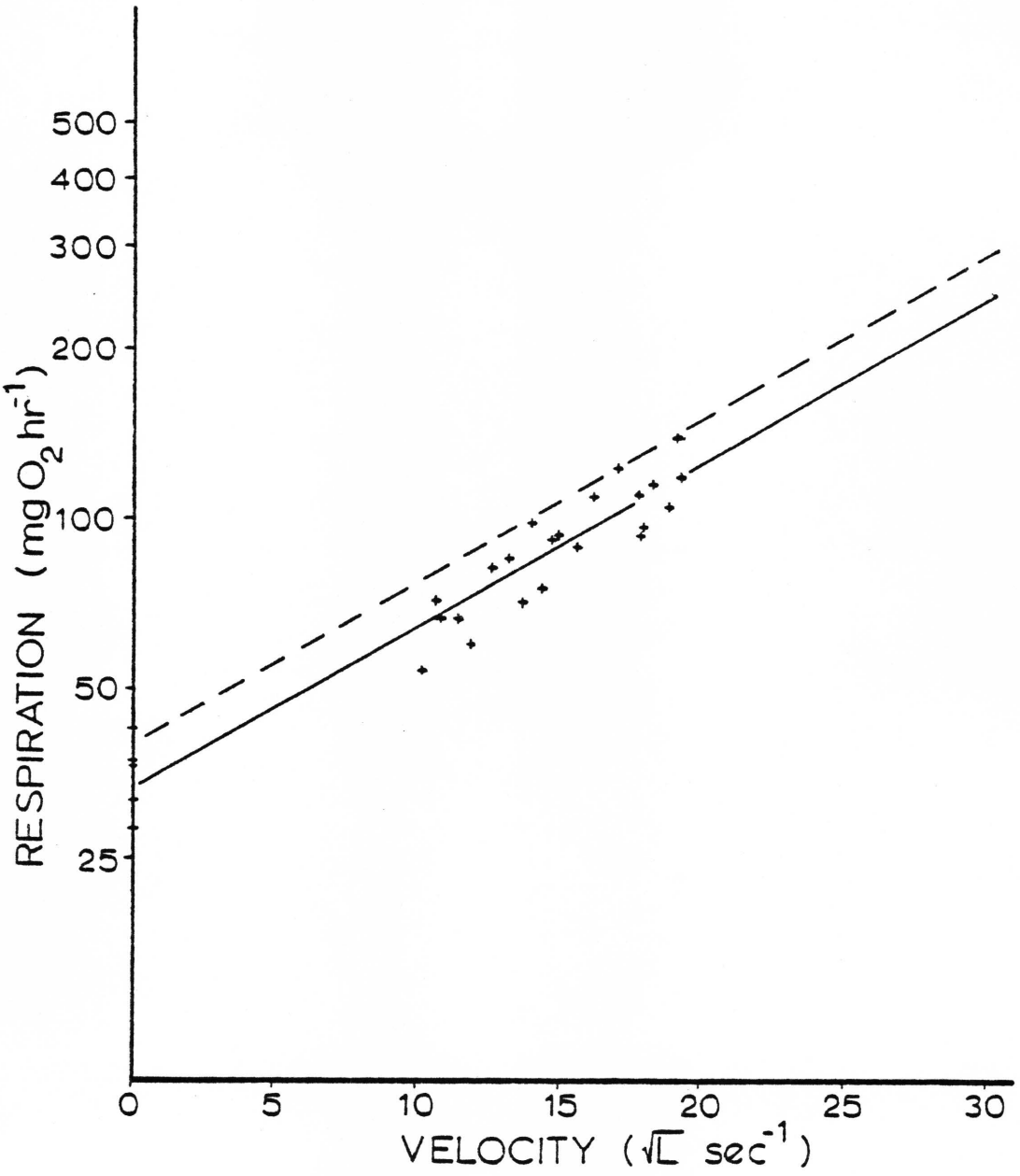
Appendix Fig. 5. Respiration and weight plot at 28C and 35 ppt. for Lutjanus campechanus in polluted (liquid phase) water. Crosses represent observed data. Solid line drawn from equation 5a in Table 1. Dashed line is for estimate of the standard level drawn parallel to the resting respiration line through the lowest measured values. Resting measurements made in flow-through chambers.



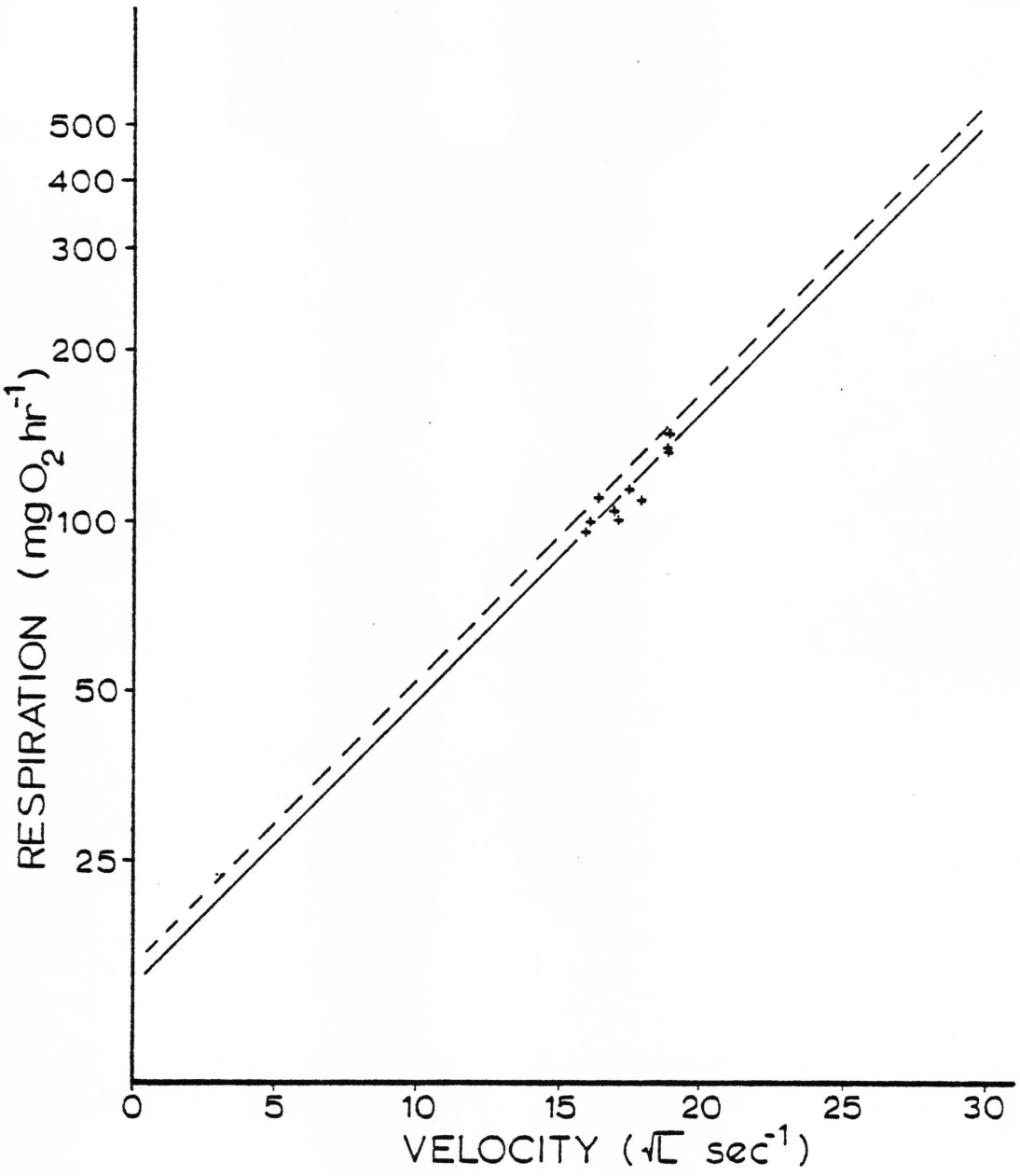
Appendix Fig. 6. Respiration and swimming velocity plot at 20C and 35 ppt. for Lutjanus campechanus in control water. Crosses represent observed data. Solid line drawn from equation 6a in Table 1. Dashed line is for estimate of maximum sustained level drawn parallel to the active respiration line through the highest measured values. Active swimming measurements made in Blazka respirometer.



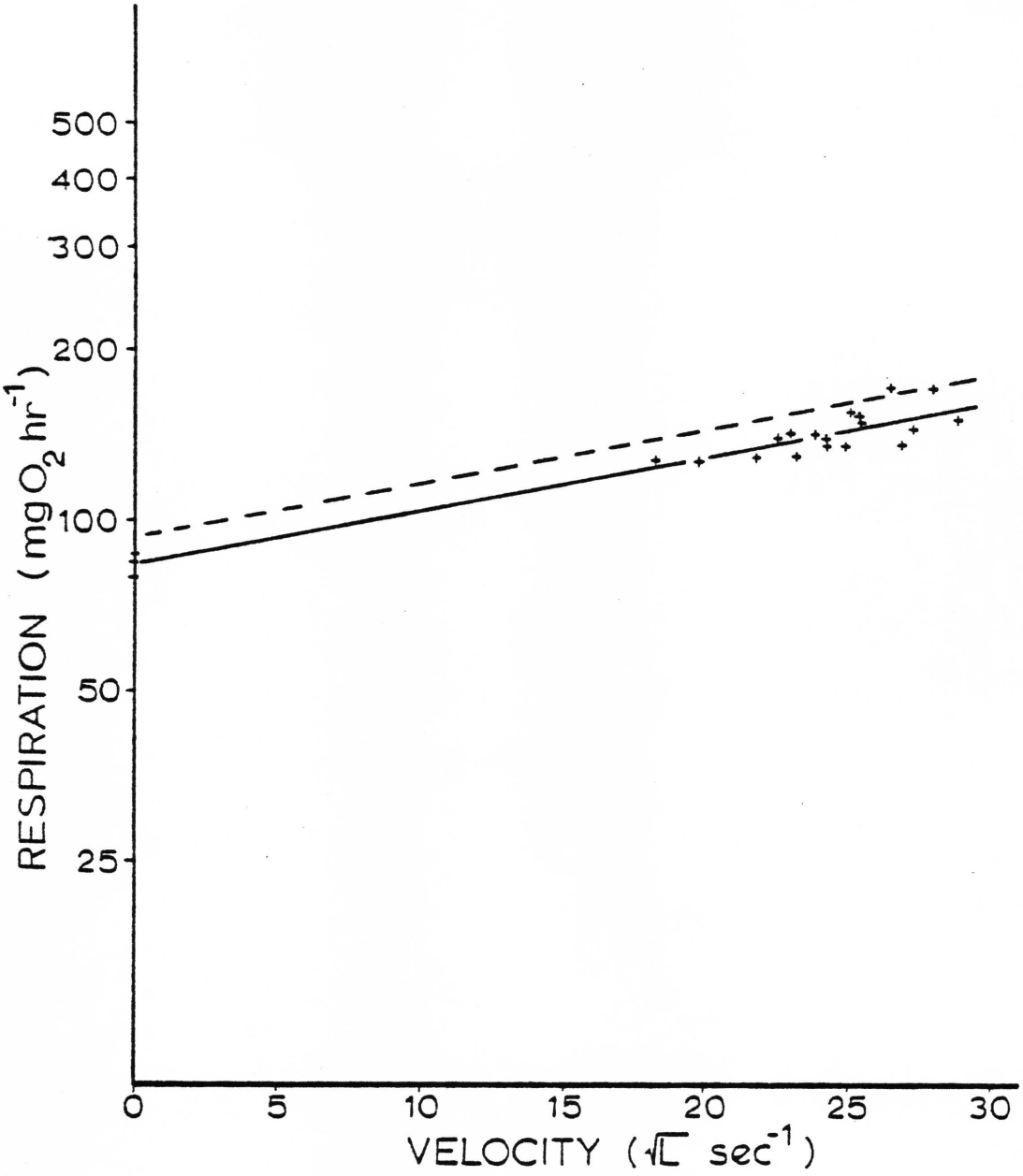
Appendix Fig. 7. Respiration and swimming velocity plot at 28C and 35 ppt. for Lutjanus campechanus in control water. Crosses represent observed data. Solid line drawn from equation 7a in Table 1. Dashed line is for estimate of maximum sustained level drawn parallel to the active respiration line through the highest measured values. Active swimming measurements made in Blazka respirometer.



Appendix Fig. 8. Respiration and swimming velocity plot at 20C and 35 ppt. for Lutjanus campechanus in polluted (liquid phase) water. Crosses represent observed data. Solid line drawn from equation 8a in Table 1. Dashed line is for estimate of maximum sustained level drawn parallel to the active respiration line through the highest measured values. Active swimming measurements made in the Blazka respirometer.



Appendix Fig. 9. Respiration and swimming velocity plot at 20C and 35 ppt. for Lutjanus campechanus polluted (sludge phase) water. Crosses represent observed data. Solid line drawn from equation 9a in Table 1. Dashed line is for estimate of maximum sustained level drawn parallel to the active respiration line through the highest measured values. Active swimming measurements made in the Blazka respirometer.



Appendix Fig. 10. Respiration and swimming velocity plot at 28C and 35 ppt. for Lutjanus campechanus in polluted (liquid phase) water. Crosses represent observed data. Solid line drawn from equation 10a in Table 1. Dashed line is for estimate of maximum sustained level drawn parallel to the active respiration line through the highest measured values. Active swimming measurements made in the Blazka respirometer.